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INHERITANCE OF PATHOGENICITY IN MELAMPSORA LINI[1]

H. H. Flor[2]

(Accepted for publication December 3, 1941)

INTRODUCTION

The flax-rust fungus, *Melampsora lini* (Pers.) Lév., is autoecious and long-cycle; that is, it produces its pycnial, aecial, uredial, and telial stages solely on species of flax. Allen (1) demonstrated the heterothallic nature of *M. lini*, and Flor (3, 4) found that rust of cultivated flax, *Linum usitatissimum* L., comprised numerous physiologic races.

Every flax variety tested has been susceptible to 1 or more of 24 physiologic races identified from North and South American collections of flax rust. However, no single race known is capable of attacking all of the 11 differential varieties used in these tests. In the northern United States, flax rust overwinters by means of telia (6) and, consequently, natural hybridization is probably coincident with the initiation of infection each year. A sound program for the production of desirable rust-resistant varieties of flax requires a knowledge not only of the interaction of the factors governing rust reaction possessed by the varieties of flax but also of the interaction of the factors for pathogenicity in various races of the flax-rust fungus. The data reported in the present paper deal with the latter subject.

Knowledge of the inheritance of pathogenicity in the rusts is limited. It was not until 1927 that Craigie (2) demonstrated the heterothallic nature of rusts and thus made possible the genetic study of these organisms. Physiologic races of rust are differentiated by the type of infection produced on a series of differential varieties, a laborious process restricting the number of progeny cultures that can be studied. Additional impediments to the genetic study of pathogenicity in the rusts are the capricious germination of the telia and the difficulty of obtaining races of known homozygosity. It also is probable that the differential varieties used to identify the physiologic races are not capable of resolving the rust into all of its genotypic strains.

New physiologic races of cereal rusts have been obtained by selfing or crossing known races of *Puccinia graminis tritici* Eriks. and Henn. (8, 12, 13, 16), *P. graminis avenae* Eriks. and Henn. (10), and *P. rubigo-vera tritici* (Eriks. and Henn.) Carl. (17), as well as by crossing the different varieties of stem rust (14). The data of Johnson, Newton, and Brown (8), obtained in a study of the inheritance of pathogenicity in a cross between two races (9a × 36) of *P. graminis tritici*, point toward Mendelian inheritance. In this cross the pathogenicity of F_2 rust cultures to Arnautka, Mindum, and Spelmar appeared to be inherited as a unit and was conditioned by a single

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

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pair of factors with virulence dominant. Pathogenicity to Kanred was conditioned by a single pair of factors with avirulence dominant, and pathogenicity to Vernal by 2 pairs of duplicate factors with avirulence dominant. Each of these 3 characters appeared to be inherited independently. In a cross between races of *P. graminis avenae*, Johnson and Newton (10) found that in the F_1 avirulence was dominant to virulence.

MATERIAL AND METHODS

The races of *Melampsora lini* here reported were of diverse origin. Races 6, 9, and 10 originated from uredial collections made in Minnesota in 1934. Race 24 was obtained as a uredial collection from North Dakota in 1938. Race 20 was derived from a telial collection obtained from Uruguay in 1935, and race 22 from telia received from Argentina in 1937. The telia material used in these studies, with the exception of that of race 24 was directly descended from the single urediospore isolate from whose pathogenic properties the original race was described. The telia of race 24 were collected in the field in 1938 on Bombay, a variety that had been immune from all other races.

Considerable difficulty has been experienced in germinating teliospores produced in the greenhouse. Best success was obtained from telia developed on greenhouse-grown plants ripening in April, stored in the laboratory at room temperature for several weeks, frozen in blocks of ice for 6 months, and then alternately wetted and dried at 2-day intervals for several weeks. Even with this technique, germination has been exceedingly erratic, the teliospores of one race failing to germinate, although developed simultaneously and receiving treatment identical with those of a race that germinated profusely.

The capricious germination of the teliospores made it desirable to determine their viability prior to inoculating flax plants with sporidia. This was done by first wetting the segments of telia-bearing flax stems for 2 to 4 hours and then placing them on a glass slide in a Petri dish, with its cover lined with moist filter paper, and incubating overnight at 55–60° F. A microscopic examination of portions of the slide adjacent to the telia for the presence of sporidia revealed the state of activity of the teliospores.

In rust studies (1, 12) the essential feature of methods employed in inoculating plants with sporidia was to permit the gametophytic sporidia to shower down on a rust-susceptible plant in a moist atmosphere. In the studies here described satisfactory results were obtained by the following technique. The flax segments bearing the germinating teliospores were placed on a wet filter paper in the cover of a Petri dish which was inverted and stuck in the top of a large bell jar by means of a ball of moist clay. The bell jar, lined with moist filter paper, was placed over a 4-inch pot containing 5 vigorously growing rust-susceptible flax plants, 8 to 12 inches high. The plants were previously atomized with tap water until numerous fine water droplets formed on the leaves. The amount of infection was roughly controlled by observing the viability of the teliospores as shown by the pre-

inoculation test and varying the quantity of telial material placed over the plants and the duration of the sporidial shower accordingly. As a rule, telial germination was poor and the plants were subjected to sporidial inoculation for 10 to 14 hours and then incubated in a moist chamber for an additional 12 hours.

Sporidial infection was first manifest as flecks on the leaves, usually 6 to 8 days after inoculation, the time varying with light and temperature conditions prevailing during the incubation period. Since cultures resulting from the transfer of pycnial nectar from one haploid infection to another comprised the basic unit in these studies, leaves having several pycnial infections were picked from the plant as soon as the pycnia were evident. Four to 6 additional days were allowed for the development of aecia from multiple infections not readily visible, before nectar was transferred from one pycnium to another. Except on heavily infected leaves, the development of normal aecia from what appeared to be single pycnial infections rarely occurred.

The amount of nectar produced by pycnia varied from almost microscopic droplets to drops so large that they dripped from the leaf. Usually, nectar production was relatively limited. The technique employed in transferring pycniospores from one haploid infection to another was as follows: A small loop of 28 gauge chromel wire was flamed, dipped in water or a solution of 5 per cent sucrose in water, and touched to a droplet of pycnial nectar. The nectar thus lifted was then transferred to a second pycnium. In attempted selfing, the usual procedure was to start at the base of the plant and transfer nectar to the lower pycnial infection from the just below the leaf bearing the infection receiving the nectar. By this pycnium immediately above it. A colored thread was tied around the stem method it was assured that pycniospores from a single pycnium would effect fertilization in not more than one aecium. This is essential in a genetic study of rusts as the paternal and maternal pustules contribute equally in Mendelian inheritance.

The aecia ruptured the epidermis of the leaf exposing the aeciospores in 2 to several days following transfer of nectar to a pycnium of opposite sex. Usually, in 5 days approximately 50 per cent of the matings produced vigorous aecia. As soon as the aecium was evident the leaf bearing it was plucked, laid on a glass slide, and the aeciospores dusted by means of a camel-hair brush on the unfolding leaves of the terminal bud of 3- to 4-inch seedlings of a rust-susceptible variety. The inoculated seedlings were atomized with tap water and incubated under bell jars for 18 to 24 hours at 55 to 60° F. The resultant urediospores were collected in glass vials and 4 to 8 seedlings of each of the rust-differentiating flax varieties were inoculated by dusting these spores on the leaves and terminal bud. The plants thus inoculated were placed in moist chambers, atomized with tap water, and incubated 24 hours. They were then removed to rust compartments in a greenhouse kept at about 70° F. during the day and 60° F. at night. To

insure vigorous growth, daylight was supplemented during the hours of darkness with artificial illumination from 200-watt Mazda bulbs hung 12 inches above the plants.

The physiologic races of flax rust have been differentiated by the type of uredia produced on 11 varieties of flax (4). Infection type may be regarded as the visible expression of the reaction of the host to the parasite, a measure of either the susceptibility of the host or of the pathogenecity of the parasite. The presentation of the results has been facilitated by the use of the terms avirulent, semivirulent, moderately virulent, and virulent to describe types of rust infection, corresponding to classes of host reaction. Classes of host reaction and types of rust infection used in this paper are as follows:

Class of host reaction		Type of rust infection		
Immune	(I)	0	No uredia evident	Avirulent
Resistant	(R)	1, 2	Uredia small with chlorosis or necrosis	Avirulent
Semi-resistant	(SR)	3–	Uredia variable with necrosis	Semi-virulent
Moderately susceptible	(S–)	1– to 3	Uredia small to medium; little necrosis, variable chlorosis, sensitive to environmental conditions	Moderately virulent
Susceptible	(S)	3 to 4	Uredia medium to large without chlorosis or necrosis	Virulent

Quantitative differences as shown by vigor of sporulation and number of pustules are indicated by plus (+) and minus (–) signs.

Resistant, semiresistant, and moderately susceptible reactions were considered to be "intermediate" in contrast with immune and susceptible.

Although hybridizing and selfing as applied to rusts may not be identical with the use of these terms as applied to spermatophytes, they are genotypically homologous. Instead of a single pollen-cell nucleus fusing with a single egg-cell nucleus, there probably occur numerous dikaryotic associations of the paternal and maternal nuclei. However, if the life history and nuclear behavior of *Melampsora lini* as reported by Allen (1) be true, all of the aeciospores, urediospores, and teliospores resulting from the transfer to one haploid pycnium of pycniospores from a second haploid pycnium of opposite sex are genotypically identical, except for possible mutations.

There are several sources of error in a genetic study of a rust such as *Melampsora lini*. It is not possible to ascertain that each pycnium is haploid and not the result of infection by two sporidia of the same sex. Also, the aecium of *M. lini* is caemoid, usually develops very rapidly, yet all aecial infections do not develop at the same rate. The aeciospores begin to shed as soon as the leaf epidermis covering the pustule ruptures and, consequently, there is the possibility of spores from one pustule falling on

another. Attempts to catch the aecium just before the epidermis ruptured resulted in poor infection, loss of many cultures, and in obtaining numerous degenerate type cultures resembling the "subepidermal strains" of Johnson and Newton (9). Mixed cultures resulting from infections by two sporidia of the same sex but carrying different factors for pathogenicity, as well as those due to accidental contamination of one aecium with spores from another, could be detected if one of the differential varieties were resistant to one and susceptible to another of the races comprising the mixture. Detection was difficult if the races involved differed in pathogenicity on a variety that was either immune or susceptible (having no intermediate infection type), since only the susceptible type of pustule would be evident. Errors due to these sources would increase the proportion of virulent races, since a mixed culture would be read as having the pathogenicity of the more virulent race.

RESULTS

Selfed Parent Races

The reactions of the 11 differential varieties of flax, employed in identifying the physiologic races of the rust fungus, to the parental urediospore cultures and to the selfed cultures of each of the 6 races that have been studied are given in table 1.

The physiologic races of *Melampsora lini* were less heterozygous than were those of *Puccinia graminis tritici* and *P. graminis avenae,* as indicated by other studies (8, 10, 13). Three of the 6 races tested, namely, races 10, 22, and 24, appeared to be homozygous, as the reaction of the 11 rust differentiating flax varieties to all of the selfed cultures of each race agreed with their reactions to the parent race. Certain selfed cultures of each of races 6, 9, and 20, differed pathogenically from the parent races. Differences in pathogenicity of the progeny of these 3 pathogenically heterozygous races were in each case confined to 1 of the 11 differential varieties. However, a different variety was involved in each instance. Of the selfed cultures of race 6, 7 were like the parent to which Akmolinsk is resistant, and 2 differed from the parent only in producing a virulent type of infection on Akmolinsk. The range of pathogenicity of these 2 cultures was unlike that of any race previously described; therefore, a new number, namely, No. 33,[3] has been assigned to them. Of the 11 selfed cultures of race 9, 8 were like the parent race to which Williston Golden is moderately susceptible, and 3 were like race 13 to which this variety is resistant. Of the selfed cultures of race 20, 15 were like the parent race from which Buda is immune. Buda was susceptible to the other 4 cultures, which agreed in range of pathogenicity with race 19. In each case, the segregating ratio approximated

[3] Race numbers 25 to 32, inclusive, have been assigned to the 8 physiologic races identified by W. Straib, (Zum epidemischen Austreten des Leinrostes in Ostpreussen. Nachrichtenbl. f. den. Deut. Pflanzenschutzdienst **19**: 49–51. 1939) from European collections of flax rust as follows: H–1, race 25; S–1, race 26; S–2, race 27; D–1, race 28; S–3, race 29; D–2, race 30; D–3, race 31; and D–4, race 32.

TABLE 1.—*Reaction of 11 differential varieties of flax to parent races of Melamspora lini and to the F_1 cultures of selfed races and hybrids*

Parent race, and selfed or hybrid F_1 culture	Number of cultures studied	Reaction of differential variety											Race of culture
		Buda C.I. 270–1	Williston Golden C.I. 25–1	Williston Brown C.I. 803–1	Akmolinsk C.I. 515–1	J. W. S. C.I. 708–1	"Pale blue crimped" C.I. 647	Kenya C.I. 709–1	Abyssinian C.I. 701	Argentine C.I. 462	Ottawa 770 B C.I. 355	Bombay C.I. 42	
Race 6		R[a]	S	S	R+	I	S−	R	I	I	I	I	6
Selfed race 6	2	R	S	S	S	I	[b]	R	I	I	I	I	33
Selfed race 6	7	R	S	S	R+	I	[b]	R	I	I	I	I	6
Race 9		S−	S	S	R+	S	S−	R	I	I	I	I	9
Selfed race 9	3	S−	R	S	R+	S	R− to S−[b]	R	I	[c]	[c]	[c]	13[d]
Selfed race 9	8	S−	S−	S	R+	S	R− to S−[b]	R	I	[c]	[c]	[c]	9
Race 10		R+	R+	R	R+	I	S−	R+	I	I	I	I	10
Selfed race 10	11	R+	R+	R	R+	I	R[b]	R	I	[c]	[c]	[c]	10
Race 20		I	S	S	S	I	S	S	S	S	I	I	20
Selfed race 20	4	S	S	S	S	I	S	S	S	S	I	I	19
Selfed race 20	15	I	S	S	S	I	S	S	S	S	I	I	20
Race 22		S	S	S	S	I	S	S	S	S	S	I	22
Selfed race 22	9	S	S	S	S	I	S	S	S	S	S	I	22
Race 24		S−	S	S	R+	I	S−	R	I	I	I	S	24
Selfed race 24	15	S−	S	S	R+	I	S−	R	I	I	I	S	24
Race 9 × 10	13	SR to S−	R	S−	R+	I	R− to S−[b]	R−	I	[c]	[c]	[c]	17
10 × 9	19	SR	R	S−	R+	I	R[b]	R	I	[c]	[c]	[c]	17[d]
Race 6 × 22	1	SR	S	S	S	I	[b]	R	R−	I	I	I	34
6 × 22	2	SR	S	S	R+	I	[b]	R	R+ to I	I	I	I	14
22 × 6	2	SR	S	S	S	I	[b]	R	R−	I	I	I	34
22 × 6	1	SR	S	S	R+	I	[b]	R	R+ to I	I	I	I	14
6 × 24	2	SR	S	S	R+	I	[b]	R	I	I	I	I	14[d]
24 × 6	2	SR	S	S	R+	I	[b]	R	I	I	I	I	14[d]
22 × 24	1	S	S	S	R+	I	[b]	R	R+ to I	I	I	I	2[d]
24 × 22	6	S	S	S	R+	I	[b]	R	R+ to I	I	I	I	2[d]

[a] Plus and minus signs indicate somewhat greater or less resistance or susceptibility than the letter designating the host reaction. The letters signify the following: I, immune; R, resistant; SR, semi-resistant; and S, susceptible.

[b] These tests were conducted during the spring months and the reaction of "pale blue crimped" is abnormal.

[c] These differential varieties were not in use when tests were made.

[d] The reaction of several of the differential varieties to these F_1 cultures differed slightly from their reactions to the type race, but, because of the effect of environment on the intermediate reactions (R+, R, R−, SR, and S−), it has been considered inadvisable to differentiate races on the basis of differences in degree of reaction.

that that would have been expected were the parent race heterozygous for a single factor pair conditioning infection type on the variety to which the selfed cultures segregated for pathogenicity.

In order to determine the pathogenic range inherent in each selfed race, a mass transfer of pycniospores was made between the pycnia on heavily infected flax plants. The differential varieties were inoculated with a mixture of the resulting aeciospores and also with the urediospore increase resulting from the inoculation of a rust-susceptible variety with the aeciospore composite. In no instance did the composite aeciospore or urediospore collection have a wider range of pathogenicity than the selfed cultures of that race. The presence of races having a more restricted range of pathogenicity than that of the parent or the selfed cultures would not be apparent when a composite inoculum is used.

F_1 HYBRIDS BETWEEN RACES

Crosses between Races 9 and 10

Williston Golden was resistant to the 32 F_1 cultures of crosses between races 9 and 10. This is of special interest because selfing studies indicated that race 9 was heterozygous for pathogenicity to this variety, the factor for moderate virulence being dominant to that for avirulence. In the hybrid, apparently the factor of race 10 for avirulence to Williston Golden was dominant to the factor of race 9 for moderate virulence.

Repeated attempts to germinate telia, produced in the greenhouse, from crosses between races 9×10[4] and 10×9 have failed. However, significance cannot be attached to this, as parallel attempts to germinate some telia of races originating from urediospores collected in the field and subjected to identical growing conditions also failed.

Crosses between Races 6 and 22

Race 6 (Fig. 1, *A*) has a restricted pathogenicity. Williston Golden, Williston Brown, and "pale blue crimped" are the only differential varieties susceptible or moderately susceptible to it. Race 22 (Fig. 1, *B*) has the widest pathogenic range of all the races thus far identified. All of the differential varieties are susceptible to it except J.W.S. and Bombay, which are immune. None of the differential varieties has an intermediate reaction to race 22.

Selfing of race 6 showed it to be heterozygous for pathogenicity to Akmolinsk and indicated that avirulence was dominant and conditioned by a single pair of factors. Selfing of race 22 showed it to be homozygous for the production of the virulent infection type on Akmolinsk. The number of cultures of the cross between race 6 and 22 was limited by the paucity of pycnia of race 6. However, Akmolinsk was susceptible to 3 and resistant

[4] Following the precedent of Newton, Johnson, and Brown (12), the race placed first (maternal) is the one on whose haploid pustules aecia are developed as the result of a transfer to them of pycniospore-containing nectar of the other (paternal) race.

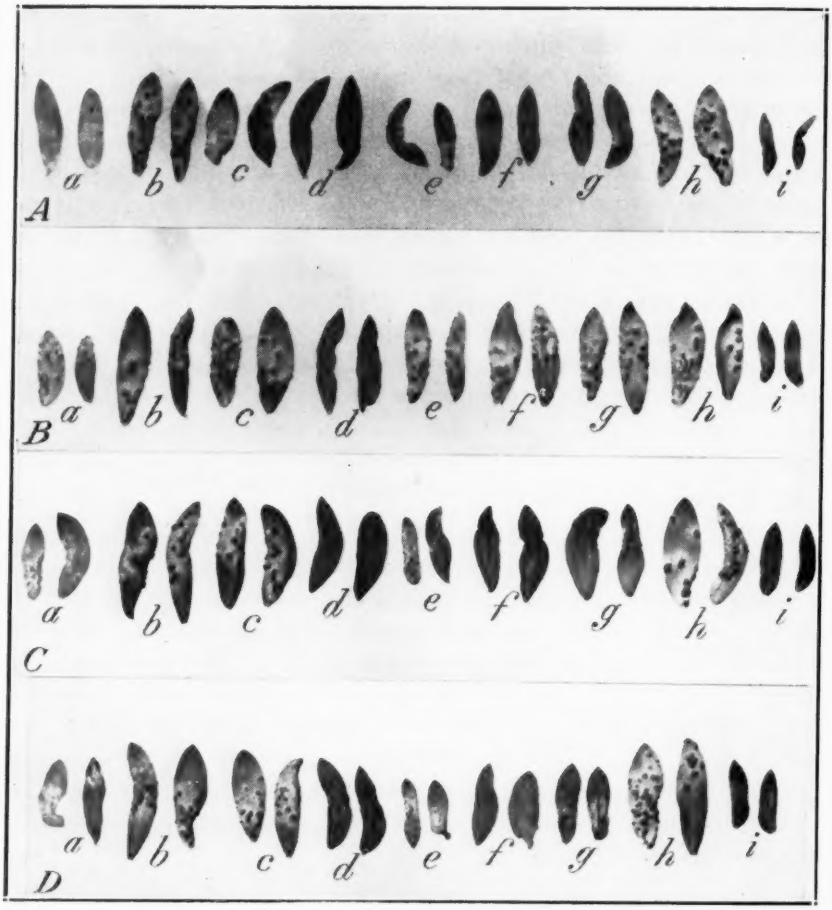

FIG. 1. Infection types produced by: *A*, race 6; *B*, race 22; *C*, F_1 of race 6 × race 22; *D*, F_1 of race 22 × race 6, on paired leaves of the flax varieties: *a*, Buda; *b*, Williston Golden; *c*, Akmolinsk; *d*, J.W.S.; *e*, Abyssinian; *f*, Ottawa 770B; *g*, Argentine; *h*, Bison, and *i*, Bombay.

to 3 of the 6 F_1 hybrid cultures. This is the ratio that would be expected if race 6 were heterozygous and race 22 homozygous for a pair of factors conditioning ability to produce a virulent infection type on Akmolinsk.

Buda was semiresistant to the 6 F_1 cultures of hybrids between races 6 and 22, a reaction intermediate to that of this variety to the parent races. The F_1 cultures of this cross were identical in pathogenicity except for the infection types on Akmolinsk and Abyssinian. Three of the 6 F_1 cultures had a range of pathogenicity resembling that of race 14 and similar to that of the F_1 hybrid cultures of race 24 × race 6 (Fig. 2, *C*). Akmolinsk was resistant and Abyssinian was highly resistant to or immune from these cultures. The other 3 cultures (Fig. 1, *C* and *D*), to which race number 34 has been assigned, had a range of pathogenicity different from that of any previously described race. To these, Akmolinsk was susceptible and Abyssinian moderately resistant.

In all tests thus far made, Abyssinian has been highly resistant to or immune from all races to which Akmolinsk has been resistant; and moderately resistant, moderately susceptible, or susceptible to races to which Akmolinsk has been susceptible. Akmolinsk has been susceptible to all races attacking Abyssinian but the latter has been resistant to certain races to which the former has been susceptible.

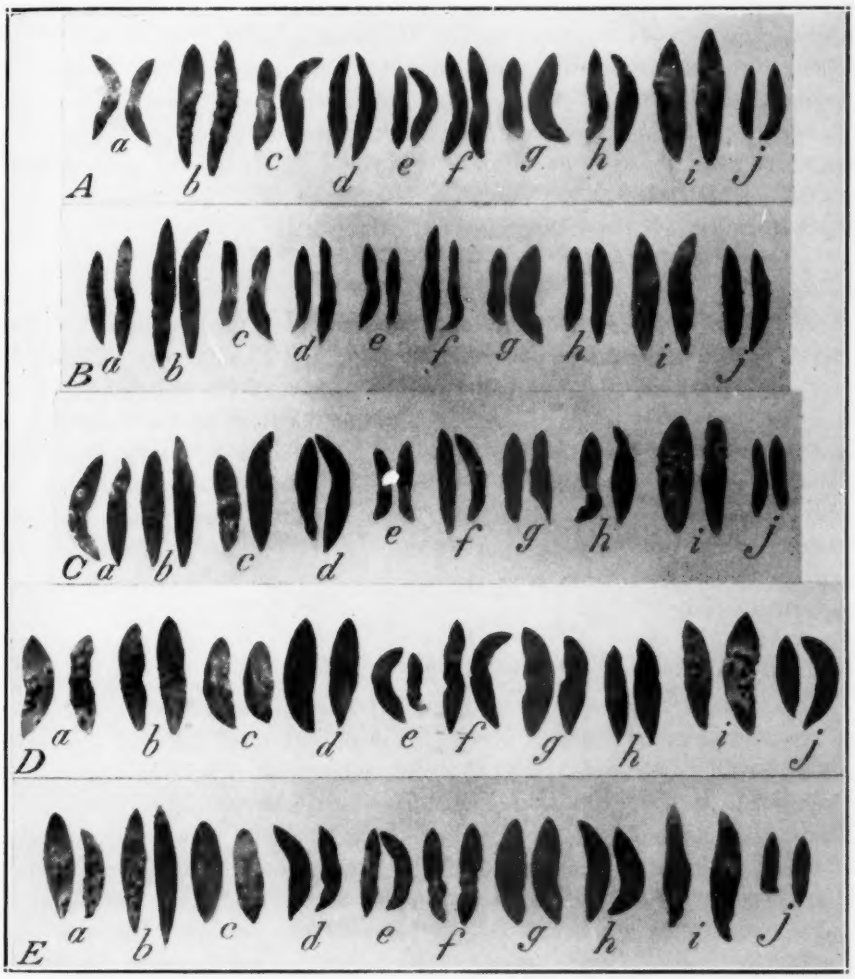

FIG. 2. Infection types produced by: *A*, race 6; *B*, race 24; *C*, F_1 of race 24 × race 6 (Race 14); *D*, F_1 of race 22 × race 24; and *E*, F_1 of race 24 × race 22, on paired leaves of the flax varieties: *a*, Buda; *b*, Williston Golden; *c*, Akmolinsk; *d*, J.W.S.; *e*, Abysinnian; *f*, Kenya; *g*, Ottawa 770B; *h*, Argentine; *i*, Bison, and *j*, Bombay.

Crosses between Races 22 and 24

Races 22 and 24 differ distinctly in pathogenicity to several of the differential varieties. All the differential varieties are susceptible to race 22 except J.W.S. and Bombay, which are immune (Fig. 1, *B*). Bombay is

susceptible, and Akmolinsk, Kenya, Abyssinian, Argentine, and Ottawa 770B are resistant to or immune from race 24 (Fig. 2, *B*).

The range of pathogenicity of the F_1 cultures conformed to the pattern of the other hybrids. Williston Golden and Williston Brown, susceptible to both parent races, were susceptible to the F_1 hybrids. Buda, moderately susceptible to race 24 and susceptible to race 22, was more susceptible to the F_1 hybrids than to the race 24 parent (Fig. 2, *D*, *E*). Akmolinsk and Abyssinian were as resistant to the F_1 hybrids as they were to race 24. J.W.S., immune from both races, also was immune from the F_1 hybrids. Argentine and Ottawa 770B, immune from race 24 but susceptible to race 22, and Bombay, immune from race 22 but susceptible to race 24, were immune from the F_1 hybrids. The F_1 hybrids had a range of pathogenicity resembling that of race 2. Thus, in these crosses, as in the preceding ones, the factors for avirulence appeared to be dominant.

Crosses between Races 6 and 24

The crosses between races 6 and 24 were made in the spring of 1939 and only 4 F_1 cultures survived over-summer storage. The parents, race 6 (Fig. 2, *A*) and race 24 (Fig. 2, *B*), differ only in pathogenicity to Buda and Bombay. These 2 varieties are, respectively, highly resistant to and immune from race 6; and moderately susceptible and susceptible, respectively, to race 24. The hybrids had the pathogenicity of race 14. As in the crosses already discussed, the reaction of Buda to the F_1 hybrid cultures was somewhat intermediate to its reaction to the parent races. The reaction of Buda to these hybrids, although approaching its reaction to race 24, was distinctly less susceptible (Fig. 2, *C*).

F_2 Cultures of Race 6 × Race 24

One of the F_1 cultures of race 6 × race 24 was selfed and the distribution of the pathogenic characters in which the parent races differed was determined for 96 F_2 cultures. The F_1 cultures segregated for pathogenicity to Akmolinsk, as well as to Buda and Bombay. As previously mentioned, although Akmolinsk was resistant to both parent races, it was susceptible to some of the selfed cultures of race 6; an indication that the parent race 6 was heterozygous for pathogenicity to this variety. Apparently, the haploid pycnium of race 6 fertilized by pycniospores of race 24, from which this hybrid culture developed, possessed the factor for virulence to Akmolinsk. The reaction of the other 8 differential varieties to the F_2 cultures of this cross was identical with their reaction to the parental and F_1 cultures.

The reaction of Akmolinsk and Bombay to all cultures of *Melampsora lini* has been clear cut. The former has been either highly resistant or susceptible and the latter either immune from or susceptible to all races with which they have been tested. The reactions of Buda to the several races are diverse, varying by almost imperceptible stages from immunity to susceptibility. Since the reaction of Buda is influenced by environment, tests with this vari-

ety were repeated. To assure uniform environmental conditions during the period of rust development, the reaction of Buda to the 96 F_2 cultures was determined simultaneously. After inoculation and incubation, the pots were placed on a greenhouse bench and rotated daily so as to subject each pot of inoculated plants to relatively uniform light conditions. When the infection types had fully developed, the entire series was arranged according to severity of infection. A natural division seemed to be into the following 5 classes, based on type and degree of infection: (1) resistant (R); (2) moderately resistant (R–), intermediate between the resistant and semiresistant classifications having pustules more numerous and vigorous than the former and smaller than those of the latter; (3) semiresistant (SR); (4) slightly susceptible (SR–) intermediate between the semiresistant and the moderately susceptible classes of host reaction, having more numerous, medium-size pustules of greater vigor than the semiresistant class but accompanied by distinct necrotic lesions; and (5) moderately susceptible (S–).

The frequency distribution of the reaction of Buda, Akmolinsk, and Bombay to the F_2 progeny of the cross between race 6 and race 24 is given in table 2. Apparently, 2 pairs of factors are involved in conditioning pathogenicity

TABLE 2.—*Segregation in the F_2 of a cross between race 6 and race 24, for pathogenicity to the varieties Buda, Akmolinsk, and Bombay*

Variety	Class of host reaction	Infection type	Number of F_2 cultures producing indicated infection type			
			Calculated		Observed	
					[a]	[b]
Buda	Resistant	1		6		5
Buda	Intermediate:					
	Moderately resistant	1 to 2+	24		24	
	Semiresistant	1, 2 and 3–	36	84	42	84
	Slightly susceptible	1, 2+ and 3–	24		18	
	Moderately susceptible	1+ to 3		6		7
						[c]
Akmolinsk	Resistant	1	72			71
Akmolinsk	Susceptible	3 to 4	24			25
						[d]
Bombay	Immune	0	72			67
Bombay	Susceptible	3 to 4	24			29

[a] X^2 for a ratio of infection types on Buda of 1 *type 1*: 4 *types 1 to 2+*: 6 *types 1, 2 and 3–*: 4 *types 1, 2+ and 3–*: 1 *types 1+ to 3* = 2.83. *P* lies between 0.50 and 0.70.

[b] X^2 for a ratio of infection types on Buda of 1 *type 1*: 14 *types 1 to 3–* (intermediate): 1 *types 1+* to *3* = 0.33. *P* lies between 0.80 and 0.90.

[c] X^2 for a ratio of infection types on Akmolinsk of 3 *type 1*: 1 *types 3 to 4* = 0.056. *P* lies between 0.80 and 0.90.

[d] X^2 for a ratio of infection types on Bombay of 3 *type 0*: 1 *types 3 to 4* = 1.39. *P* lies between 0.20 and 0.30.

to Buda. On this basis it is assumed that avirulence is obtained in the segregates in which the two dominant factors are homozygous and moderate virulence when the 2 recessive factors are homozygous and intermediate types when either or both pairs of factors are heterozygous. Thus the ob-

served segregating ratio of the 96 F_2 cultures for pathogenicity to Buda closely approaches the theoretical 1:14:1, *P* lying between 0.80 and 0.90. Although division into the intermediate reaction classes was arbitrary, the observed ratio of segregates approached that which would be expected if ability to cause Buda to have (1) a moderately resistant reaction (R–) was conditioned by 3 dominant and 1 recessive factors, (2) a semiresistant reaction (SR) by 2 dominant and 2 recessive factors, and (3) a slightly susceptible reaction (SR–) by 1 dominant and 3 recessive factors, *P* lying between 0.50 and 0.70.

The segregating ratios obtained for pathogenicity to Akmolinsk and Bombay satisfactorily fit expectation if the virulent infection type is conditioned, in each instance, by an independent pair of recessive factors, *P* for Akmolinsk lying between 0.90 and 0.95, and *P* for Bombay lying between 0.20 and 0.30.

The factors conditioning virulence to Akmolinsk appeared to be linked with one of the pairs of factors conditioning avirulence to Buda. To the 25 F_2 cultures to which Akmolinsk was susceptible, Buda was either resistant, moderately resistant, or semiresistant. In table 3 are given the theoretical distribution of genotypes and the observed segregation for classes of reaction, assuming that single independent pairs of recessive factors condition ability to produce the virulent type of pustule on Akmolinsk and Bombay and that two pairs of quantitative factors condition the infection type on Buda with very close or complete linkage between the factors conditioning virulence to Akmolinsk and one of the pairs of factors conditioning avirulence to Buda.

The infection types of 1 of the 96 F_2 cultures did not conform to the assumed complete linkage between a resistant infection type on Buda and a susceptible infection type on Akmolinsk. Theoretically, both Buda and Akmolinsk should not be resistant to any of the F_2 cultures. Actually, one such culture did occur. This may be accounted for by crossing over, by mutation, or by failure to express normal infection type because of environment. Since Buda was distinctly resistant to this culture in both tests, the crossing over or the mutation theories seem more plausible. Ignoring this one culture, the *P* value for observed segregation for pathogenicity according to theoretical genotypes was about 0.50, indicating good agreement of the data with the hypothesis.

The occurrence of intermediate classes of reaction on Buda, together with its sensitiveness to changes in environment makes this variety somewhat unsatisfactory as a rust differential. The following classes of reaction of Buda have served to differentiate physiologic races of the flax-rust organism: (1) Resistant (R), including moderately resistant (R–) and highly resistant (R+); (2) semiresistant (SR); and (3) susceptible (S), including moderately (S–) and highly susceptible (S+). When F_2 cultures to which Buda is slightly susceptible (SR–) are grouped with those to which it is semiresistant, the actual distribution of physiologic races comprising the 96 F_2 cultures closely approaches the theoretical, *P* lying between 0.50 and 0.70.

TABLE 3.—*Genotypes and segregation for*

TABLE 3.—*Genotypes and segregation for pathogenicity to the flax varieties Buda, Akmolinsk, and Bombay, and for physiologic races in the F_2 of a cross between races 6 and 24*

Assumed genotypes[a]	Reaction to F_2 culture of			Number of cultures having indicated genotype based on pathogenicity		Physiologic race number	Distribution of physiologic races	
	Buda	Akmolinsk	Bombay	Calculated	Observed[b]		Calculated	Observed[c]
F_2 Genotype:								
AABC/Be(C)Dd(D)	R	R	I	0.0	1	6	9.0	9
AABe/bCDd, AABe/bCDD	R–	R	I	6.0, 3.0 = 9.0	8			
AAbC/bCDd, AAbC/bCDD, AaBe/bCDd, AaBe/bCDD	SR	R	I	3.0, 1.5, 12.0, 6.0 = 22.5	26	14	40.5	37
AabC/bCDd, AabC/bCDD, aaBe/bCDd, aaBe/bCDD	SR–	R	I	6.0, 6.0, 3.0, 6.0, 3.0 = 18.0	11			
aabC/bCDd, aabC/bCDD	S–	R	I	3.0, 1.5 = 4.5	3	2	4.5	3
AABe/BeDd, AABe/BeDD, AaBe/BeDd, AaBe/BeDD	R–	S	I	3.0, 1.5 = 4.5; 6.0, 3.0 = 9.0	2, 11	33	13.5	13
aaBe/BeDd, aaBe/BeDD	SR	S	I	3.0, 1.5 = 4.5	5	34	4.5	5
AABe/bCdd, AaBe/bCdd	R–	R	S	3.0	3	35	3.0	3
AAbC/bCdd, AabC/bCdd, aaBe/bCdd	SR	R	S	6.0, 1.5 = 7.5; 3.0, 3.0 = 6.0	8, 7	36	13.5	15
aabC/bCdd	S–	R	S	1.5	4	24	1.5	4
AABe/Bedd	R	S	S	1.5	2	37	4.5	4
AaBe/Bedd	R–	S	S	3.0	2			
aaBe/Bedd	SR	S	S	1.5	3	38	1.5	3

[a] Assumed genotype: parent gamete of race 6, ABeD; of race 24, abCd; and of F_1 culture, AaBbCeDd.
[b] $x^2 = 12.02$, P lies between 0.50 and 0.70. [c] $x^2 = 6.767$, P lies between 0.50 and 0.70.

The data in table 3 can be explained on a factorial basis by assuming that the haploid pustule of race 6 contributed the quantitative factors AB conditioning avirulence to Buda, the recessive factor c conditioning virulence to Akmolinsk, and the dominant factor D conditioning avirulence to Bombay, and the pycniospores from the haploid pustule of race 24 contributed the quantitative factors ab, conditioning moderate virulence to Buda, the dominant factor C conditioning avirulence to Akmolinsk, and the recessive factor d conditioning virulence to Bombay, with linkage between the factors Bc and bC. The 10 physiologic races theoretically possible on the basis of this assumption were obtained, of which 6, namely races 33, 34, 35, 36, 37, and 38, had not been isolated prior to these studies.

CYTOPLASMIC INHERITANCE

Some evidence of cytoplasmic inheritance of certain pathogenic characters has been reported in crosses between certain races of *Puccinia graminis tritici* (13) and *P. graminis avenae* (10). In those cases certain pathogenic characters of the maternal parent, which could not be accounted for on a strictly Mendelian basis, were evident in the F_1 and subsequent generations. In the present studies, in addition to making crosses in both directions, pycnial nectar was exchanged between single haploid pustules of the races hybridized. Only in the cross between races 9 and 10 was there any indication of cytoplasmic inheritance. Buda appeared to be slightly more susceptible to F_1 cultures of 9×10 than to those of 10×9.

DISCUSSION

The development of rust-resistant varieties of flax is complicated by the existence of numerous physiologic races of the flax-rust fungus. The physiologic races occurring in different regions differ in range of pathogenicity. For instance, Punjab was susceptible in Minnesota and North Dakota in 1940 but has been immune from rust in Argentina, while Ottawa 770B is susceptible in Argentina (15) but has been immune in North America (4). The physiologic races prevalent in a given locality may differ from season to season. In 1938 and 1940 Bombay was susceptible to rust at Fargo, N. Dak., but in 1939 this variety was immune from the physiologic races occurring there. Some of the fundamental problems in the study of a plant disease, such as flax rust, are the determination of (1) the range of pathogenicity of the causal organism, (2) if and how new strains come into existence, (3) the potential range of pathogenicity of these new strains, and (4) the interaction between factors for pathogenicity in the pathogen and those for resistance in the host. These problems involve genetic studies of the pathogen as well as of the host varieties that exhibit a differential response to the physiologic races of the pathogen. The determination of the pathogenic capacities of the selfed and hybrid cultures that have been presented in this paper is a preliminary step in such a study.

The determination of the interaction of factors for pathogenicity in the

flax-rust fungus and those for resistance in the host is complicated by the occurrence of numerous pathogenically distinct physiologic races of the former as well as by the existence of numerous strains of the latter possessing distinct factors for resistance. It is known that a single factor in J.W.S. conditions immunity from at least 18 physiologic races of *Melampsora lini* and that other flax varieties possess several independent factors for immunity from or resistance to specific physiologic races (5, 11). At least several of the factors for immunity and resistance appear to occur in allelomorphic series. Consequently, it has not been possible to obtain a strain of flax homozygous for certain combinations of the resistant and immune factors. It would be of value to know if parallel conditions exist in the pathogen. Is the ability of a virulent race to attack a number of flax varieties, known to possess different factors for rust reaction, due to one or to a number of factors each of which overcomes a specific "resistance" factor in the host? If numerous pathogenic factors are involved may not some of them be allelomorphic?

The data in the present paper indicate that the range of pathogenicity of a physiologic race of *Melanspora lini* is determined by pathogenic factors specific for each resistance factor possessed by the host. Henry (7) reported that in Bombay immunity from North American collections of flax rust was conditioned by a single pair of factors. In the present studies, pathogenicity to Bombay was conditioned by a single pair of factors with avirulence dominant. Unpublished data of the writer show that the reaction of Akmolinsk to the physiologic races used in these studies is conditioned by a single pair of factors. Pathogenicity to Akmolinsk of the selfed and hybrid cultures also was conditioned by a single pair of factors, avirulence being dominant, independent of the factors for pathogenicity to Bombay.

Flor (5) found that 2 pairs of factors were involved in conditioning reaction of Buda to rust. Both pairs were operative as incomplete dominants or quantitative factors to races to which Buda was resistant, while only one of the factor pairs was operative to race 20, from which Buda was immune. Selfing studies of race 20 indicated that it was heterozygous for pathogenicity to Buda and that the virulent type of infection was recessive and conditioned by a single pair of factors. To the F_1 hybrids between races to which it was susceptible and those to which it was resistant, Buda produced an intermediate infection type indicative of a lack of dominance of the factors for pathogenicity. The segregating ratio of the F_2 cultures of such a hybrid indicated that 2 pairs of quantitative factors conditioned pathogenicity to Buda.

SUMMARY

The inheritance of pathogenicity in *Melampsora lini* was studied by selfing and hybridizing physiologic races.

Three of the six physiologic races selfed, namely races 10, 22, and 24, were apparently homozygous for pathogenicity, the infection types of each

selfed culture being identical with those of its parent on the 11 differential flax varieties. The three other races, 6, 9, and 20, were heterozygous, some selfed cultures of each race differing from the parent in pathogenicity when tested on the differential varieties.

Avirulence was invariably dominant in crosses between physiologic races, although, on Buda, dominance was incomplete. Consequently, the F_1 hybrid resembled the weaker of the two parents in pathogenicity or combined the less virulent characteristics of each. The range of pathogenicity of a uredial culture does not necessarily indicate its genotype. The F_1 uredial cultures of a cross between the virulent races 22 and 24 had the pathogenicity of race 2, a race of relatively restricted pathogenic range.

The F_2 cultures of a cross between race 6 and race 24 segregated for infection types on Buda, Akmolinsk, and Bombay. The segregation for pathogenicity of these cultures was explained by assuming that, in this hybrid, 2 pairs of quantitative factors conditioned infection type on Buda, that single independent pairs of factors, with avirulence dominant, conditioned infection types on Akmolinsk and Bombay, and that the pair of factors conditioning infection type on Akmolinsk was linked with one of the pairs of factors for pathogenicity to Buda. The 10 physiologic races theoretically possible from the assumption were isolated. Six of these had not been described previously.

The pathogenic characters of physiologic races obtained by selfing and hybridizing could be accounted for by Mendelian segregation and recombination of pathogenic characters inherent in the parent races.

Pathogenicity of the selfed and hybrid cultures to Akmolinsk and Bombay, varieties in which single pairs of factors determine reaction to rust, was conditioned by single pairs of independent factors. Pathogenicity to Buda, a variety possessing 2 pairs of factors for resistance to race 6, was conditioned by 2 pairs of factors in the hybrid between race 6 and race 24. Only 1 of the 2 pairs of factors in Buda is effective in conditioning rust reaction to race 20, which was heterozygous for pathogenicity to this variety. The selfed cultures of race 20 segregated for pathogenicity in a single factorial ratio when tested on Buda. These facts suggest that the pathogenic range of each physiologic race of the pathogen is conditioned by pairs of factors that are specific for each different resistant or immune factor possessed by the host variety.

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STUDIES ON THE PATHOGENICITY OF FUSARIUM SPECIES ASSOCIATED WITH ROOT ROT OF WHEAT[1]

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INTRODUCTION

A root disease of wheat and other cereals caused by *Helminthosporium sativum* P. K. and B. and *Fusarium* spp., and usually referred to as common root rot, is very prevalent and destructive in the three prairie provinces of Canada. Recently, Simmonds (31) gave a good description of the symptoms of this disease, and called attention to its economic importance in Western Canada.

Although *Helminthosporium sativum* is by far the most important fungus associated with common root rot of small grain crops, particularly of wheat and barley, *Fusarium culmorum* (W. G. Sm.) Sacc., and several other species of *Fusarium*, have been consistently isolated from root-rotted specimens of cereals and grasses. For instance, Simmonds (28), Greaney and Bailey (14), and Broadfoot (7) report that, in Western Canada, the crowns and basal parts of wheat plants are frequently infected with *Fusarium* spp. Bolley (6) and Atanasoff (1) claim that many different species of *Fusarium* cause root rot of wheat and other cereals in the United States. Bennett (3, 4) isolated and identified 14 different species of *Fusarium* from British cereals. Russell (22), in 1930, and Samuel and Greaney (24), in 1935, obtained several species of *Fusarium* from the roots and stem bases of wheat plants collected from different fields in England. Shen (27) reported that brown foot rot of cereals, caused by *Fusarium* spp., was unusually prevalent in certain districts of England in 1938. According to Gordon and Sprague (12) one of the most common genera of fungi associated with gramineous root rots in the Northern Great Plains of the United States is *Fusarium*.

From the studies of Waksman (32), Beckwith (2), Henry (17), Reinking and Manns (20, 21), Sadasivan (23), and others, it would appear that fungi belonging to the genus *Fusarium* are regularly occurring soil inhabitants. Recent studies on the microflora of the soil carried out at this Laboratory have shown that these fungi are extremely common in grain soils. According to Gordon (11), species of *Fusarium* accounted for 10.8 per cent of 13,800 isolations of fungi obtained from the soil of permanent grain plots

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at Winnipeg in 1936. In 1937, they comprised 18.2 per cent of the total isolations. Many species, varieties, and forms, belonging to 8 sections of the genus *Fusarium*, were represented.

Plant disease surveys and field experiments carried out for a number of years in Western Canada have indicated that the incidence of Helminthosporium-Fusarium root rot of wheat varies greatly from year to year. The cause of this variation has been attributed to such factors as the occurrence of physiologic forms of the pathogens, differences in soil and climatic conditions, and the association effects of other soil-inhabiting microorganisms. Garrett (9) has given an adequate review of the literature on factors affecting the pathogenicity of cereal root- and foot-rot fungi, and the results of earlier studies on this subject in Canada have been summarized by Simmonds (29, 30).

Many different species of *Fusarium* usually are associated with seedling blight and root rot of wheat. Obviously, therefore, a knowledge of the parasitic capabilities of these different fungi is the essential basis for the development of permanent control measures. Furthermore, for the effective control of Fusarium root rot of wheat and other cereals it is important to have a thorough knowledge of the conditions that favor the development of parasitic species of *Fusarium* in the soil, and influence the susceptibility of their host plants.

The objects of the present experiments were (i) to determine the relative virulence of different cereal isolates of *Fusarium*, particularly *F. culmorum*, on wheat, and (ii), to study the influence of soil moisture, soil temperature, and of certain common soil-inhabiting fungi on the pathogenicity towards wheat in the seedling stage of the 5 species of *Fusarium* most frequently isolated from the roots and crowns of cereal plants and from grain soils in Manitoba.

MATERIALS AND METHODS

A list of the species of *Fusarium* and isolates of *F. culmorum* used in the investigation is given in table 1, which contains also the origin of the fungi and the date of their isolation.

Greenhouse pot tests were used to study the pathogenicity of the above-mentioned fungi to wheat seedlings. Inoculum of each organism was prepared by growing the fungus in flasks on a mixture of sterilized sand and cornmeal (2 per cent) for about 15 days. This sand-cornmeal inoculum was then mixed with autoclaved soil, 1 part of inoculum to 30 parts of soil, by weight, and the soil mixture placed in four 6-inch flower pots. Before planting, the soil in each pot was brought to a uniform moisture content of 50 per cent of its total water-holding capacity and weighed. This moisture content was maintained throughout the period of the experiment by weighing the pots at 2-day intervals and adding enough sterile water to bring them back to their original weight. In order to allow the fungus to become well established in the soil, 3 days were allowed to elapse between the time of soil inoculation and date of planting.

Twenty-five seeds of Marquis wheat were planted per pot. Before planting, the seed was surface-sterilized and washed in sterile water. It was then inoculated with a water suspension of spores of the fungus to be tested. The control seed for each experiment was dipped in sterile water. After planting, the pots of each experiment were randomized on a large bench in the centre of the greenhouse.

In order to investigate the effect of soil moisture, soil temperature, and other soil-inhabiting fungi on the pathogenicity of *Fusarium* species to wheat seedlings, Marquis wheat was inoculated with *F. culmorum* (W. G. Sm.)

TABLE 1.—*Source of species and isolates of Fusarium*

Organism	Isolate No.	Year isolated	Host	Locality
Fusarium redolens	26	1935	Wheat	Morden, Man.
" *oxysporum*	73	1937	"	Winnipeg, Man.
" *avenaceum*	66	1936	"	" "
" *equiseti*	94	1937	"	" "
Fusarium culmorum	19A	1930	"	Saskatoon, Sask.
	19B	"	"	" "
	19C	"	Oats	" "
	38	1932	Wheat	Rhodes, Man.
	39	"	"	" "
	529	1933	Brome Grass	Winnipeg, Man.
	854	1935	Barley	Emerson, Man.
	856	"	Wheat	Bowsman, Man.
	937	"	"	Pilot Mound, Man.
	1040	"	"	Brandon, Man.
	1111	"	"	Clearwater, Man.
	1119	"	Oats	Melita, Man.
	1122	"	Wheat	Deloraine, Man.
	1158	"	"	Binscarth, Man.
	1193	"	"	Foxwarren, Man.
	1197	"	"	Virden, Man.
	1202	"	"	Shoal Lake, Man.
	1263	"	"	Bowsman, Man.
	1280	"	"	Griswold, Man.
	1300	"	"	Pipestone, Man.
	6	1936	"	Pope, Man.
	10	"	"	Harpenden, Eng.
	15	"	"	New Norway, Alta.
	285	"	"	Saskatoon, Sask.

Sacc., *F. redolens* Wr., *F. avenaceum* (Fr.) Sacc., *F. oxysporum* Schl. v. *aurantiacum* (Lk.) Wr., and *F. equiseti* (Cda.) Sacc., respectively, in 6-inch pots of soil. The methods used to inoculate the seed and soil were essentially similar to those described above. Additional methods, introduced to meet the requirements of these specific experiments, will be described in later sections of this paper.

In all greenhouse experiments, germination was usually complete 12 days after planting, at which time the number of emerged plants per pot series was counted. The seedlings were harvested and the degree of infection on each plant was recorded 4 to 5 weeks after planting. The infection classes and the disease rating formula used in these studies were the same as those employed by Greaney (15) in previous pot tests with *Fusarium culmorum*.

After the disease data had been recorded, the total green weight per pot series was taken.

In addition to seedling tests, studies were made to determine the relative pathogenicity of the above-mentioned species of *Fusarium* to adult wheat plants. The methods used for this purpose were briefly as follows. Surface-sterilized seeds of Marquis wheat were sown in sterilized quartz sand. When the seedlings were 6 days old they were transplanted from the sterilized sand to 6-inch pots of soil containing inoculum of the fungus to be tested. At the time of transplanting the entire root system of the young seedlings was inoculated by dipping it in a water suspension of spores and mycelial fragments of the respective fungi. Five inoculated seedlings were then placed in each of 5 pots of inoculated soil. Thereafter, the soil of each fungus series was inoculated with a spore suspension at regular intervals (10, 20, 50, and 70 days) during the growing period. The moisture and temperature of the soil were maintained at fairly constant levels in all pots throughout the experiment. The plants were harvested at maturity and the weight of plants and yield of grain recorded. The intensity of disease on each plant, and on the plants of each fungus series, was recorded according to the methods described by Greaney *et al.* (16). The experiment was run at 2 different times.

A field experiment was conducted in 1939 to determine the relative virulence on mature wheat plants of *Fusarium redolens*, *F. avenaceum*, *F. oxysporum* and *F. equiseti*, and 9 selected isolates of *F. culmorum*. Surface-sterilized seed of Renown wheat was inoculated with spores of the fungus to be tested, and planted in plots of artificially infested soil. The complete experiment consisted of 4 replicates of 16 plots each. Data on emergence were taken 23 days after planting, and notes on the incidence of disease were taken just before the plants ripened. One rod row of each plot was harvested for yield data. The methods used to infect the soil and record the amount of root-rot infection on individual plants were those employed in earlier field studies with cereal root-rot fungi (16).

The plant emergence, disease, and yield data of each greenhouse and field experiment were treated statistically by the analysis of variance method, and the significance of the observed differences between organisms and soil treatments determined.

EXPERIMENTAL RESULTS

Pathogenicity of Species and Isolates of Fusarium

Greenhouse Experiments. The results of greenhouse experiments designed to determine the relative pathogenicity to wheat seedlings of 24 different isolates of *Fusarium culmorum* are given in table 2. The data of both seedling and adult plant pathogenicity tests with 5 different species of *Fusarium* are summarized in table 3. To economize space, the complete analyses of variance data for these greenhouse experiments are not given. For each experiment, however, the results of the analyses showed highly significant differences between organisms for all of the properties measured.

It is evident from table 2 that a wide variation exists in the ability of isolates of *Fusarium culmorum* to attack young wheat plants. In view of the great susceptibility of wheat seedlings growing in sterilized re-infested soil to root-rot fungi, the present test must be considered a severe one. Nevertheless, even under the most favorable conditions for infection, the great majority of the 24 isolates tested were either non-pathogenic or only weakly pathogenic to Marquis wheat. One isolate of *F. culmorum*, No. 38,

TABLE 2.—*The relative pathogenicity to Marquis wheat seedlings of different isolates of Fusarium culmorum*

(Data are means of 4 trials)

Isolate of *Fusarium culmorum*[a]	Percentage of plants emerged	Disease rating	Green weight of plants (grams)
38	47.0	64.0	42.6
937	59.8	35.9	60.9
1263	57.2	35.8	63.2
19C	68.5	34.2	62.5
1300	63.0	32.2	68.5
1202	66.0	28.3	61.0
10	61.8	27.8	68.5
1040	59.2	25.8	62.4
1119	63.0	25.1	67.3
854	61.2	24.6	66.0
1280	63.0	24.4	70.0
6	64.0	23.8	69.0
15	67.5	22.9	74.6
1158	66.2	22.4	71.6
1193	65.0	21.1	66.7
856	60.2	20.6	67.6
1111	67.2	19.5	71.2
1197	78.5	17.6	84.0
19B	64.5	16.8	73.3
19A	75.2	16.4	78.0
1122	72.2	16.0	76.7
39	74.0	11.9	78.3
285	77.0	10.0	79.0
529	70.0	6.0	80.3
Control A	88.7	2.8	91.2
Control B	90.6	3.0	92.1
Necessary difference, 5% level	10.8	10.1	13.3

[a] Isolates arranged in order of virulence.

exhibited extreme virulence, while a few others, including Nos. 937, 1263, and 19C, were moderately pathogenic. The present results are in agreement with those of Sanford and Broadfoot (26), and show that the great majority of the isolates of *F. culmorum* obtained from cereal plants and from grain soils are weakly, if at all, pathogenic to young wheat plants.

In the final analysis of this experiment, disease ratings and total green weights for the plants of the individual fungus series were correlated. The significance of the coefficient obtained was determined by the method described by Goulden (13). A highly significant correlation coefficient of -0.9259 was obtained. This result indicated that root-rot infection was very

closely associated with plant growth, the higher the degree of infection the poorer was the development of the young wheat plants.

It is evident from table 3 that, of the 5 species of *Fusarium* tested, only *F. culmorum* (Isolate No. 38) was markedly pathogenic to Marquis wheat in both the seedling and post-seedling stages of plant growth. *F. avenaceum* and *F. equiseti* were nonpathogenic, or only very weakly pathogenic, to

TABLE 3.—*The relative pathogenicity to seedling and adult Marquis wheat plants of different species of Fusarium*

(Data are means of 2 trials)

Organism	Seedling test			Adult plant test		
	Plant emergence	Disease rating	Green weight	Disease rating	Green weight	Grain yield
	Per cent		*Grams*		*Grams*	*Grams*
F. culmorum	40.1	88.0	9.7	59.0	88.4	11.1
F. avenaceum	79.6	9.5	62.8	32.4	113.2	15.0
F. equiseti	78.0	6.5	63.9	26.8	124.4	16.7
F. oxysporum	81.6	5.2	64.8	18.8	119.8	18.7
F. redolens	82.3	4.6	63.8	15.6	125.6	19.2
Control	82.2	4.8	65.2	13.6	126.1	22.3
Necessary difference, 5% level	9.9	12.9	10.1	11.2	12.6	6.1

wheat seedlings, but attacked plants in the post-seedling stage of growth to a moderate degree. On the other hand, *F. oxysporum* and *F. redolens* were nonpathogenic to both seedling and post-seedling Marquis wheat plants. In both experiments, the intensity of root infection was negatively associated with plant growth and yield.

Field Experiment. A field experiment was made in 1938 to determine the relative pathogenic capabilities of *Fusarium avenaceum, F. equiseti, F. oxysporum, F. redolens,* and 9 isolates of *F. culmorum* to adult wheat plants. The isolates of *F. culmorum* used gave, when tested under greenhouse conditions, a wide range in degrees of virulence on wheat seedlings (Table 2). For the field tests, different seed lots of wheat were inoculated with the respective isolates and fungi by the spore-suspension method. Immediately after inoculation the seed was planted in plots of artificially infested soil.

The analyses of variance for the plant emergence, disease, and yield data

TABLE 4.—*Analysis of variance for plant emergence, disease rating and yield. (Winnipeg Field Experiment, 1938)*

Source of variance	Degrees of freedom	Mean square		
		Plant emergence	Disease rating	Yield
Replicates	3	535.19[a]	149.24[a]	146.44[a]
Organisms	15	983.26[a]	54.96[b]	108.91[a]
Error	45	38.28	25.44	20.45

[a] Exceeds mean square error, 1% level of significance.
[b] Exceeds mean square error, 5% level of significance.

of the experiment are given in table 4. It is evident from this table that highly significant differences between organisms were obtained for all properties measured.

TABLE 5.—*Relative pathogenicity of five species of Fusarium, and of several isolates of Fusarium culmorum to Renown wheat under field conditions in 1938*

(Data are means of 4 plots)

Organism	Isolate No.	Percentage of plants emerged	Disease rating	Yield per acre (bushels)
F. equiseti	94	62.4	39.0	32.7
F. avenaceum	66	52.2	38.6	35.0
F. redolens	26	64.7	36.9	34.6
F. oxysporum	73	61.3	36.7	36.3
F. culmorum	937	37.5	43.4	28.4
	19A	28.6	43.2	27.4
	529	30.8	41.4	24.2
	1263	28.8	41.1	22.4
	38	39.3	41.0	25.0
	285	34.1	40.3	26.3
	10E	35.3	39.2	26.5
	19B	53.1	38.0	26.3
	15	49.8	36.9	24.3
	19C	48.2	34.6	34.1
Control—A	.....	66.6	31.0	36.9
Control—B	.....	67.1	30.9	38.1
Necessary difference, 5% level	.....	9.3	7.6	6.8

In table 5 are given field-plot data for the different organisms. It is evident that, of the species tested, *Fusarium avenaceum* and *F. equiseti* were only slightly pathogenic to adult plants of Renown wheat, while the amount of infection on plants inoculated with *F. redolens* and *F. oxysporum* was not significantly different from that on the uninoculated control plants. The field results with *F. avenaceum*, *F. equiseti*, *F. redolens* and *F. oxysporum* were in accordance with the greenhouse results with these fungi (Table 3). On the other hand, the field results with different isolates of *F. culmorum* were not in agreement with the earlier greenhouse tests (Table 2). For instance, some of the isolates that proved to be distinctly pathogenic to post-seedling plants in the field were only weak parasites on wheat seedlings under greenhouse conditions, while others that showed a very low degree of virulence on adult plants in the field were strongly pathogenic to young wheat plants in the greenhouse tests. Further studies are required to determine the nature and cause of the variability observed in the parasitic behavior of different isolates of this important cereal root-infecting fungus.

Influence of Soil Moisture and Temperature on the Pathogenicity of Fusarium Species

Greenhouse pot experiments were conducted to determine the effect of soil moisture and soil temperature on the pathogenicity of *Fusarium culmorum* (Isolate 38), *F. equiseti*, *F. avenaceum*, *F. redolens*, and *F. oxysporum*.

Soil Moisture. Two experiments were made to determine the influence of moisture on pathogenicity. At the commencement of each experiment the soil in one half of the pots of each fungus series was adjusted to 60 per cent of its moisture-holding capacity (high moisture series), while the soil in the remainder of the pots was adjusted to a moisture-holding capacity of 40 per cent (low moisture series). High and low soil moisture pots of each fungus series were placed in each of 4 thermostatically controlled temperature tanks maintained at 10, 15, 20, and 25 degrees C., respectively. The respective soil moistures were maintained throughout the experiment.

The results of both soil-moisture experiments are summarized in table 6. To economize space, the complete analyses of variance for plant emergence, disease rating, and green weight of plants are not given. For each experiment, however, the results of these analyses showed that there were no moisture effects. The interactions of moistures and temperatures, and moistures and organisms, were also insignificant. On the other hand, significant differences between organisms were shown for all properties studied. In both experiments, temperature effects were significant for disease rating and green weight, but not for plant emergence.

TABLE 6.—*The influence of soil moisture on the pathogenicity of species of Fusarium to Marquis wheat seedlings (Data are means of four different temperatures—10, 15, 20, 25° C., and of 2 trials)*

Experiment	Organism	Percentage of plants emerged		Disease rating		Green weight of plants (grams)	
		Moisture content of soil[a]					
		High	Low	High	Low	High	Low
I	*F. culmorum*	37	40	85.7	88.1	6.7	8.8
	F. equiseti	47	48	3.8	5.8	50.8	52.4
	F. avenaceum	48	48	9.0	9.3	49.8	54.0
	Control	50	52	4.3	4.8	50.1	50.1
	Mean	45.5	47.0	25.7	25.8	39.4	41.3
II	*F. culmorum*	34	30	81.4	88.4	3.8	2.8
	F. redolens	54	54	13.6	6.6	27.8	29.0
	F. oxysporum	51	51	12.4	7.5	27.3	28.3
	Control	53	52	9.4	6.2	27.9	29.2
	Mean	48.0	46.8	29.2	27.2	22.0	22.3

[a] Differences between soil-moisture contents insignificant statistically.

The evidence presented in table 6 shows that soil moisture did not significantly increase or decrease the virulence of any of the species of *Fusarium* studied. In experiments I and II, however, there was a general tendency for low soil moisture to increase the virulence of these fungi. The results in table 6 show that *F. culmorum* (Isolate 38) was exceedingly pathogenic to Marquis wheat seedlings at both the low and high soil-moisture contents.

Soil Temperature. To ascertain the influence of temperature on the

pathogenicity of different species of *Fusarium*, 2 experiments, each consisting of 3 organisms and a control, were carried out. The fungus *F. culmorum* was included in both experiments, and each experiment was run at 4 different times. The soil for each fungus series was inoculated in the usual manner and placed in sixteen 6-inch pots. Four pots of this soil were planted with 120 inoculated seeds of Marquis wheat and placed in each of 4 temperature tanks maintained at 10, 15, 20, and 25 degrees C., respectively.

The disease rating and green-weight data of the soil-temperature experiments are given in tables 7 and 8. These tables also give the temperature data recorded for the low-moisture series of the above-mentioned soil moisture experiments.

TABLE 7.—*The influence of temperature on the pathogenicity of different species of Fusarium to Marquis wheat seedlings. Results of Soil Temperature Experiments I and II, and Soil Moisture Experiments I and II*

Degree of Seedling Infection (Disease Rating)

Experiment	Organism	Temperature ° C.				Necessary difference between temperatures[a]
		10°	15°	20°	25°	
Temperature I[b]	*F. culmorum*	72.5	87.4	88.0	89.2	6.8
	F. equiseti	2.4	4.6	7.8	4.1	
	F. avenaceum	5.1	7.8	9.5	14.3	
	Control	2.8	4.1	4.8	7.4	
	Mean	20.7	26.0	27.2	29.2	3.4
Temperature II[b]	*F. culmorum*	78.9	88.4	83.8	84.3	9.9
	F. redolens	1.4	9.3	11.2	16.4	
	F. oxysporum	1.5	7.4	14.1	16.0	
	Control	1.3	6.9	11.8	12.1	
	Mean	20.8	28.0	30.2	32.2	5.0
Moisture I[c]	*F. culmorum*	66.2	87.5	89.4	90.2	6.6
	F. equiseti	2.2	4.2	8.1	8.5	
	F. avenaceum	2.9	7.0	10.0	17.4	
	Control	2.2	3.6	5.1	8.2	
	Mean	18.4	25.6	28.2	31.1	3.3
Moisture II[c]	*F. culmorum*	86.4	88.8	92.4	86.0	11.7
	F. redolens	1.2	5.2	6.7	13.2	
	F. oxysporum	1.4	4.4	11.6	12.5	
	Control	0.8	6.2	7.3	10.6	
	Mean	22.2	26.2	29.5	30.6	5.8

Necessary difference, 5% level of significance, between organisms: Temperature Experiment I = 6.8; Temperature Experiment II = 9.9; Moisture Experiment I = 6.6; Moisture Experiment II = 11.7.

[a] 5% level of significance.

[b] Data are means of 4 trials.

[c] Data are means of low soil moisture series, and of 2 trials.

The analysis of variance results of the data of each temperature and moisture experiment established that the effects of temperature on the pathogenicity of the species of *Fusarium* tested were very great. Although temperature did not influence significantly the percentage of plants that

emerged from the soil, it had a very significant effect on the incidence of disease, as represented by the disease rating, and on the resulting green weight of the plants. In these experiments, as in previous pathogenicity tests, highly significant differences in the percentage of plants emerged, amount of disease, and total green weight of plants were observed between the species of *Fusarium* tested. There were no interactions between temperatures and organisms in any of the experiments.

TABLE 8.—*The influence of temperature on the pathogenicity of different species of Fusarium to Marquis wheat seedlings. Results of Soil Temperature Experiments I and II, and Soil Moisture Experiments I and II*

Green Weight of Plants (Grams)

Experiment	Organism	Temperature ° C.				Necessary difference between temperatures[a]
		10°	15°	20°	25°	
Temperature I[b]	*F. culmorum*	6.1	7.1	9.7	8.1	18.6
	F. equiseti	29.0	51.6	63.9	62.0	
	F. avenaceum	29.6	55.4	62.8	59.8	
	Control	30.5	50.1	65.2	59.6	
	Mean	23.8	40.1	50.4	47.4	9.3
Temperature II[b]	*F. culmorum*	4.6	2.6	4.4	3.6	8.6
	F. redolens	22.6	34.5	31.3	25.1	
	F. oxysporum	20.7	33.2	32.4	24.9	
	Control	23.1	32.7	32.8	25.7	
	Mean	17.8	25.8	25.2	19.8	4.3
Moisture I[c]	*F. culmorum*	9.5	9.3	9.1	7.4	21.6
	F. equiseti	32.4	54.2	65.1	57.8	
	F. avenaceum	34.0	60.0	66.6	55.4	
	Control	33.8	53.6	67.0	56.1	
	Mean	27.4	44.3	52.0	44.2	10.8
Moisture II[c]	*F. culmorum*	4.1	2.3	1.8	3.1	9.2
	F. redolens	22.5	35.2	36.3	21.9	
	F. oxysporum	21.1	35.2	35.0	22.0	
	Control	23.3	33.5	36.0	24.2	
	Mean	17.8	26.6	27.3	17.8	4.6

Necessary difference, 5% level of significance, between organisms: Temperature Experiment I = 18.6; Temperature Experiment II = 8.6; Moisture Experiment I = 21.6; Moisture Experiment II = 9.2.

[a] 5% level of significance.

[b] Data are means of 4 trials.

[c] Data are means of low soil moisture series, and of 2 trials.

From table 7 it is evident that temperature had a very marked effect on the virulence of the species of *Fusarium* studied. The mean-temperature figures for organisms indicate that, in all experiments, the greatest amount of injury to Marquis wheat seedlings occurred at the higher temperatures, that is, at 20° and 25° C. On the other hand, plant growth was considerably more vigorous at 20° C. than at 25° C. (Table 8). Thus, it may well be that the effect of temperature on the host is more important than its effect on the fungus. The extensive investigations of Dickson (8), McKinney

(19), and others, have indicated that young wheat plants are blighted most severely by root-rotting fungi at temperatures that serve to predispose the plants to disease, that is, relatively high temperatures for wheat. The evidence presented in tables 7 and 8 substantiates the conclusions of Dickson and McKinney, and indicates that temperature is an exceedingly important factor influencing the occurrence of seedling blight and root rot of wheat caused by *Fusarium* species.

Influence of Other Soil-Inhabiting Fungi on the Pathogenicity of Fusarium Species

Two greenhouse pot experiments were made to determine the effects of the association of certain common soil-inhabiting fungi on the pathogenicity of *Fusarium redolens*, a very weak pathogen, and on a virulent isolate (No. 38) of *F. culmorum* (Table 3). In these experiments, wherever association effects were studied, the total quantity of inoculum incorporated into the soil was double the amount used where the fungus was tested singly. The common soil-inhabiting fungi employed were *Pyronema confluens* (Per.) Tul., *Trichoderma lignorum* (Tode) Harz, *Aspergillus flavipes* (B. and S.) Thom. and Church, and *Penicillium intricatum* Thom. These fungi were originally isolated from the soil of permanent grain plots at Winnipeg, Man. In experiment I, *P. confluens* and *T. lignorum* were tested against *F. culmorum*, while *A. flavipes* and *P. intricatum* were tested against *F. culmorum* and *F. redolens* in experiment II.

In experiments I and II, 4 pots of each fungus, or combination of fungi, were prepared, planted, and placed in temperature tanks maintained at 10° and 20° C. The soil-moisture content of each pot was held at 50 per cent of the total water-holding capacity of the soil throughout the experiment. The plants were harvested at 28 days, and the disease and yield data recorded in the usual way. An adequate number of control pots were included in each experiment, and both experiments were repeated 4 times.

The analyses of variance results of both experiments showed that a high degree of significance could be attached to the differences observed in percentage of plants emerged, amount of disease, and yield, between the various organisms and combinations of organisms studied in each experiment. As might be expected from the results of the temperature experiments described in the preceding section, significant temperature effects were shown for both disease rating and green weight of the plants, but not for plant emergence.

The summarized results in table 9 show the marked virulence of *Fusarium culmorum* (Isolate 38), and the weak parasitic action of *F. redolens*, on wheat seedlings. The important point in these experiments is the fact that the presence of the fungi *Pyronema confluens* and *Trichoderma lignorum* in soil, suppressed, to an appreciable degree, particularly at 10° C., the virulence of *F. culmorum*. On the other hand, the presence of *Penicillium intricatum* and *Aspergillus flavipes* in the soil had no influence on the

pathogenicity of *F. culmorum*. The pathogenicity of *F. redolens* was not influenced by the presence of *P. intricatum* and *A. flavipes* in the soil.

The results of Experiments I and II indicate the marked effect temperature has on the pathogenicity of species of *Fusarium*. In both experiments, *F. culmorum*, whether alone or in association with other soil-inhabiting fungi, was much more virulent at 20° C. than at 10° C.

TABLE 9.—*The effect of certain common soil-inhibiting fungi on the pathogenicity of Fusarium culmorum and Fusarium redolens to Marquis wheat seedlings*

(Data are means of 4 trials)

Organisms added to soil	Percentage of plants emerged		Disease rating		Green weight of plants (grams)	
	10°	20°	10°	20°	10°	20°
Experiment I						
Fusarium culmorum	17	16	86.1	91.3	1.6	4.4
Pyronema confluens	48	50	3.4	2.9	28.5	73.6
Trichoderma lignorum	48	48	2.8	3.3	25.6	81.1
F. culmorum + *P. confluens*	28	21	73.6	84.1	3.2	6.0
F. culmorum + *T. lignorum*	25	23	77.7	87.0	3.3	2.3
P. confluens + *T. lignorum*	48	48	2.4	2.4	27.7	66.5
Control	49	48	1.9	2.2	26.2	88.5
Necessary difference, 5% level	9.6	9.6	8.0	8.0	12.6	12.6
Temperature mean	37.6	36.3	35.4	39.0	16.6	46.1
Necessary difference, 5% level, between temperature means	[a]		3.0		4.8	
Experiment II						
Fusarium culmorum	28	19	59.1	88.3	4.7	2.8
Fusarium redolens	51	49	1.2	2.6	15.5	34.6
Aspergillus flavipes	51	52	1.6	2.5	14.4	36.0
Penicillium intricatum	46	50	1.0	3.3	13.6	37.1
F. culmorum + *A. flavipes*	24	20	61.2	86.0	3.7	3.5
F. culmorum + *P. intricatum*	28	19	61.0	89.6	4.2	3.0
F. redolens + *A. flavipes*	50	50	0.6	1.9	13.2	36.5
F. redolens + *P. intricatum*	52	49	0.7	2.3	14.0	36.2
Control	51	50	0.9	1.9	14.4	34.2
Necessary difference, 5% level	12.8	12.8	9.8	9.8	9.5	9.5
Temperature mean	42.3	39.8	20.8	30.9	10.8	24.8
Necessary difference, 5% level, between temperature means	[a]		3.3		3.2	

[a] Difference insignificant statistically.

In general, the data presented in table 9 emphasize the importance of the reaction of one fungus upon another in the soil, and confirm the experiments of Henry (18), Sanford and Broadfoot (25), Garrett (10), Bisby, James, and Timonin (5), and others, who found that infection of wheat seedlings by cereal root-rotting fungi was suppressed by the antagonistic effect of certain other soil fungi and bacteria.

DISCUSSION

In this paper additional evidence is provided to support the view that certain common soil-inhabiting fungi have a marked influence on the de-

velopment of cereal root-rot fungi. It was found that infection of wheat seedlings with a virulent isolate of *Fusarium culmorum* alone, or in association with certain other common soil-inhabiting fungi, increased with a rise in temperature from 10° to 20° C. However, the conclusions of Garrett (9) and others suggest that the results observed in the present association studies represent not the true effect of temperature upon infection with *F. culmorum*, but a combination of this with the action of temperature upon biological antagonism of the soil microflora. In the present studies the antagonism of *Pyronema confluens* and *Trichoderma lignorum* to *F. culmorum* was greater at 10° than at 20° C. The work of Garrett (9), however, has shown that the antagonism of other soil bacteria and fungi to certain root-rot fungi of cereals increases with a rise in temperature to 30° C. In view of these conflicting results it is apparent that more detailed experiments are needed to determine the true effect of soil temperature upon the antagonism of other soil fungi to the root-rot fungi of cereals.

The present studies have demonstrated that the seedling stage in wheat is a very critical one from the standpoint of root rot caused by species of *Fusarium*. The primary roots of young wheat plants appear to be very susceptible to invasion and injury by virulent strains of these fungi. These experiments have further shown that the onset and progress of the Fusarium disease is greatly enhanced by weakened plant growth. Thus, the data provided by the investigation are considered sufficient to justify the conclusion that any farm practice that will stimulate early, rapid, and vigorous plant growth will be an effective means of reducing the losses due to seedling blight and root rot of wheat caused by species of *Fusarium*.

SUMMARY

Greenhouse experiments were made to determine the pathogenicity to wheat of 24 isolates of *Fusarium culmorum* (W. G. Sm.) Sacc., and of 4 other species of *Fusarium*, namely, *F. redolens* Wr., *F. oxysporum* Schl. v. *aurantiacum* (Lk.) Wr., *F. avenaceum* (Fr.) Sacc. and *F. equiseti* (Cda.) Sacc. The species of *Fusarium* studied were those most commonly isolated from root-rotted specimens of wheat, and from grain soils collected in Manitoba. Of the isolates of *F. culmorum* tested only one was distinctly pathogenic to both seedling and adult wheat plants. Under greenhouse conditions, no marked pathogenicity was exhibited by the isolates of *F. redolens*, *F. oxysporum*, *F. avenaceum* and *F. equiseti* studied. Field tests with these fungi gave essentially similar results.

The influence of soil moisture and soil temperature on the virulence of different species of *Fusarium* on wheat seedlings was studied by means of greenhouse pot tests. The moisture content of the soil did not influence significantly the virulence of the *Fusarium* species tested. On the other hand, intensity of infection with these fungi increased with an increase in soil temperature. The isolate of *F. culmorum* studied was distinctly pathogenic at 10, 15, 20, and 25° C. *Fusarium avenaceum* was pathogenic, but

weakly so, only at 25° C., while *F. redolens, F. oxysporum* and *F. equiseti* were not pathogenic at any of the temperatures employed.

In greenhouse pot tests, *Trichoderma lignorum* Tode (Harz.) and *Pyronema confluens* (Pers.) Tul., two common soil inhabitants, suppressed, to an appreciable degree, the pathogenicity of a virulent isolate of *F. culmorum*, while *Penicillium intricatum* Thom, and *Aspergillus flavipes* (B. and S.) Thom and Church, did not. The virulence of *F. redolens* was not influenced by the presence of *P. intricatum* and *A. flavipes* in the soil.

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CROSS INOCULATIONS WITH ISOLATES OF FUSARIA FROM COTTON, TOBACCO, AND CERTAIN OTHER PLANTS SUBJECT TO WILT[1]

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INTRODUCTION

Burley tobacco, *Nicotiana tabacum* L., was grown for the first time in 1938 in several counties of the Piedmont section of South Carolina, and wilting occurred in several fields. Tissue cultures of affected plants invariably gave a species of *Fusarium*. Since there was no previous record that tobacco had been grown in these fields and, since cotton, *Gossypium hirsutum* L., was known to wilt in most of them, cross-inoculation experiments were undertaken with the Fusaria from these hosts.

Wilt of coffeeweed or wild senna (*Cassia tora* L.[2]) was noted in an experimental cotton wilt plot at Allendale, South Carolina, in 1937 and similar plants were later found at 2 other locations in the State. Isolations from plants collected at all these locations yielded Fusaria similar in cultural appearances. These observations suggested that the cotton wilt Fusarium, besides existing as a saprophyte in the soil, might attack weeds and thus be perpetuated in a living host other than cotton. Therefore, cross inoculations with these Fusaria were made.

Several strains of wilt-resistant cowpeas, *Vigna sinensis* Endl., were grown on a plot known to be heavily infested with the cotton-wilt organism, but the presence of the cowpea-wilt organism in this soil had not been established. The nonresistant cowpeas used as checks were severely damaged by wilt, consequently cross inoculations of cotton and cowpeas with the fusarium-wilt organisms from these hosts were included in the present studies.

Several early publications refer to *Fusarium vasinfectum* Atk. as the cause of wilt of okra, *Hibiscus esculentus* L., without presenting adequate experimental evidence to support these statements.[3] Hence, okra and its fusarium isolate were also used in these experiments. Since wilts of tomato and watermelon are generally distributed throughout the State, tomato (*Lycopersicon esculentum* Mill.) and watermelon (*Citrullus vulgaris* Schrad.) and their fusarium isolates were included in the experiments after it had been discovered that the cotton-wilt fungus causes wilt in several other plants.

[1] Contribution of the Department of Botany and Bacteriology in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Technical contribution No. 86, South Carolina Agricultural Experiment Station.

[2] *Emelista tora* (L.) Britton and Rose, according to Small.

[3] After this paper was submitted for publication, it was discovered that Taubenhaus had performed cross inoculations with wilt Fusaria from cotton, okra, cowpeas, watermelon, tomato, cabbage, squash, and sweetpea. Taubenhaus, J. J. Wilts of watermelon and related crops. Tex. Agr. Exp. Stat. Bull. 260, 50 pp. 1920.

METHODS AND MATERIALS

The solution-culture infection technique (3) employed in other studies of cotton wilt was used chiefly in these studies. As a check on the solution-culture method, tobacco and cotton also were grown in pots of soil, each inoculated with a wheat-oats mixture of the fungal isolates from one of the several hosts. The procedure was the same as that described in other experiments by the senior writer and associates (2). The isolate of the fungus from cotton had been used in other tests and was known to be strongly pathogenic. In addition to the fusarium culture isolated from tobacco in South Carolina, 1 from Kentucky[4] and 3 from Maryland[5] also were used in the experiments. A susceptible and a resistant variety of Burley tobacco, Burley 5 and Burley 31, respectively, and the Gold Dollar variety of flue-cured tobacco were grown, but only the susceptible Farm Relief 2 variety of cotton was used. All flue-cured varieties of tobacco grown in South Carolina are apparently highly resistant to the fusarium-wilt organism in the soils of the State, since the disease is seldom seen in the fields. The cultures of the Cassia-, cowpea-, okra-, and watermelon-wilt organisms were obtained in South Carolina.

For the solution-culture method, all seed, except that of tobacco, was placed directly in the excelsior seed bed of the trays, as previously described (3). This procedure was impossible with the small seed of tobacco, so these were planted in flats of steamed soil. When the seedlings were 4 to 6 inches tall they were transferred to both soil and solution cultures. For inoculations of tobacco in soil, 2 plants were grown in each 2-gal. glazed pot, the transplanting being done 24 days after the wheat-oats inoculum was mixed with the soil.

Plants were removed from trays or pots as wilting occurred. Approximately an inch section was cut from the base of each stem, sterilized in a 3 per cent solution of sodium hypochlorite[6] for 5 minutes, and plated on 2 per cent potato dextrose agar. At the end of an experiment all remaining plants were treated similarly.

EXPERIMENTAL RESULTS

Cross Inoculations of Cotton and Cassia tora

The first seed of Cassia was collected early in the fall from plants showing symptoms of wilt injury; but the fact that these had survived to seed production indicated a resistance to the wilt fungus. The second lot of seed was gathered late in the fall probably after the seed from wilt-injured plants had been shed, and at a time when only the vigorous, resistant, and later-maturing plants were available. The very low percentages of infection from plants of the second lot (Table 7) indicate that these were characterized by very high wilt resistance. Observations in the field also support the idea that most plants are very resistant, since only a small percentage of stems

[4] Cultures supplied by W. D. Valleau.
[5] Cultures supplied by E. A. Walker.
[6] The commercial product, BK, was used.

showed internal blackening at the base. Thus in heavily infested soil it may be that natural selection has eliminated the susceptible plants. A year later a third collection of seed was made earlier in the season from plants showing very definite external symptoms of wilt, and the plants produced from this seed were the most wilt-susceptible of the 3 lots (Table 1, exp. 3).

TABLE 1.—*Inoculations of Cassia tora and cotton in solution cultures with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cassia tora				
Cassia fungus (exp. 1)	169	20.7	33.7	30.8
S. C. cotton fungus (exp. 1)	175	3.4	6.8	17.1
S. C. tobacco fungus (exp. 1)	166	6.0	12.6	24.7
S. C. tobacco fungus (exp. 3)	88	42.0	42.0	22.7
Uninoculated check (exp. 1)	96	0.0	7.3	9.4
Uninoculated check (exp. 3)	91	0.0	0.0	2.2
Cotton, Farm Relief 2				
Cassia fungus (exp. 1)	137	27.0	24.1	31.4
Cassia fungus (exp. 3)	78	98.7	98.7	82.0
S. C. cotton fungus (exp. 1)	150	88.0	86.7	82.0
S. C. tobacco fungus (exp. 1)	142	92.2	91.5	88.0
Uninoculated check (exp. 1)	64	6.2	10.9	17.2
Uninoculated check (exp. 3)	80	0.0	0.0	1.2

That *Cassia tora* is subject to infection by a species of *Fusarium* obtained from this host seems evident from the results reported in table 1, exp. 1. The percentages of infection, whether based on external symptoms, internal symptoms, or isolations of a *Fusarium*, are not high but are in the expected range for a resistant variety. Such low percentages of infection were obtained in the second experiment (Table 7) that very definite statements are not warranted from these results. As indicated above, however, the seed for this experiment probably was collected from highly resistant plants. There was slight internal blackening of a few plants and fusaria were obtained similar in appearance to the original cultures.

The results presented in table 1 indicate that *Cassia tora* is also subject to infection by the wilt fungi from cotton and tobacco. Since the percentages of infection were low in the first 2 tests, a third collection of Cassia seed was made. It will be seen in table 1, exp. 3, that 42 per cent of the plants inoculated with the South Carolina tobacco fungus showed external symptoms of wilt. The plants from which this collection of Cassia seed was made produced seed less abundantly than nearby healthy plants, but the fact that the diseased plants survived would place them in a class resistant to wilt.

It should be noted that if infection experiments on Cassia with the fungi from cotton and Cassia had been confined to the plants derived from the

second seed lot, the conclusion would have been that this weed is probably not a host for the cotton-wilt fungus (Table 7). On the other hand, the results obtained with the third collection of seed (Table 1, exp. 3) would indicate that this weed is more highly susceptible to wilt caused by the *Fusarium* species common to it and cotton and tobacco in South Carolina than field observations show.

Attention is called to the infection that occurred in the check trays of Cassia and cotton (Table 1). Elliott (8), Taubenhaus and Ezekiel (17), Crawford (7), and Kulkarni (12) have stated that some of the seed from cotton plants infected with the fusarium-wilt organism may carry the fungus internally. Nothing is known about the presence of the fungus internally in *Cassia tora,* but this is a possibility, since the seed used in the first and third experiments (Table 1) came from wilting plants. However, not all the infection in the checks of the first experiments may have come from this source, since experience with the solution-culture method has indicated that accidental infections can be avoided only by meticulous care in every detail of handling the cultures. It is believed that the weekly changing of the solution in which the trays were lifted from the bench and the solution emptied into a sink was the chief cause of trouble. Fewer infections occurred in the checks when the technique was gradually improved, so that trays were not handled, the solution was drained through sterilized syphons, new solution was added through sterilized glass tubes, and sterilized rubber gloves were worn and changed after handling the cultures inoculated with any single isolate.

Experience gained in culturing many thousands of plants indicated that a Fusarium could not always be recovered from a darkened stem, but one might be obtained from a fairly high percentage of plants of resistant varieties that show neither external nor internal symptoms of wilt. In many of the severe cases of wilting, the plants were all but dead before they were removed and cultured. In such cases fast-growing contaminating organisms made the recovery of the Fusarium difficult.

It is recognized, also, that obtaining a Fusarium from a stalk is no absolute assurance that the wilt fungus has been recovered, even though the isolate be similar in appearance to the parent culture. Tests would be necessary to prove its pathogenicity, but the great amount of labor and equipment involved make this procedure impracticable. However, those tests that have been made with a limited number of isolations indicated that the error from this source was small. *Fusarium moniliforme* frequently is encountered if a dozen or more stalks are being cultured; but its growth characteristics are distinct from the wilt-producing forms, thus usually making unnecessary any microscopic examinations to distinguish this organism. In one check solution culture, 36 per cent of the stalks gave *F. moniliforme,* but there was no wilt, and the plants were growing vigorously.

That cotton is subject to infection by the fusarium-wilt organism obtained from *Cassia tora* is evident from the results presented in tables 1, 2,

and 7. In the solution-culture experiments, 27 per cent (Table 1, exp. 1), 98.7 per cent (Table 1, exp. 3) and 100 per cent (Table 7) of the plants showed external symptoms of wilt. In a soil-culture experiment (Table 2), 100 per cent of the plants also showed symptoms of wilt. The Cassia fungus also caused wilt in the susceptible Burley tobacco (Table 2). This fungus has not been used in an infection experiment with okra, but successful infections would be expected. Smith (14) reported that . . . "Mr. Orton has found the fungus on James Island, South Carolina, in a weed, the *Cassia obtusifolia* L.[7]" and that the external and internal symptoms of wilt were

TABLE 2.—*Inoculations of cotton and tobacco in soil with the Fusarium from Cassia tora*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
Cassia fungus	31	100.0	100.0	100.0
Uninoculated check	9	11.1	11.1	11.1
Tobacco, Burley 5				
Cassia fungus	31	87.1	87.1	96.8
Uninoculated check	8	25.0	25.0	50.0

identical with those on cotton and cowpea. The observations were not supported by experimental evidence as to the connection of "the fungus" with the disease.

Cross Inoculations of Cotton and Tobacco

In these experiments cotton has been inoculated in solution culture only, with Fusaria isolated from cotton and tobacco in South Carolina, from tobacco in Kentucky, and 3 cultures from tobacco in Maryland. Other experiments (2) have shown that several cotton isolates gave the same relative rating as to pathogenicity for cotton in solution culture as in soil, but higher percentages of wilting have occurred in the former.

TABLE 3.—*Inoculation of cotton in solution cultures with the fusarium-wilt fungus of tobacco from Kentucky*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
Ky. 627 tobacco fungus	281	91.8	90.4	59.8
Uninoculated check	100	0.0	2.0	2.0

[7] Synonym of *Cassia tora* L.

The data of tables 1 and 3 show that the susceptibile Farm Relief variety of cotton inoculated with the South Carolina cotton fungus, the South Carolina tobacco fungus, and the Kentucky 627 tobacco fungus showed external symptoms of wilt in the inoculated plants of 88, 92.2, and 91.8 per cent, respectively. The same variety of cotton, inoculated with the South Carolina cotton fungus at a later date, showed 100 per cent of the plants with external symptoms of wilt (Table 7). The isolates of Fusarium from tobacco grown in South Carolina and Kentucky clearly produce wilting of cotton as readily as a virulent isolate from cotton. The tobacco isolates from Maryland, however, failed to infect the Farm Relief variety of cotton, as is noted below.

Tables 4 and 5 present the data obtained from inoculating the tobacco varieties, susceptible Burley 5, resistant Burley 31, and flue-cured Gold Dollar with 1 cotton- and 2 tobacco-fusarium isolates. Burley 5 inoculated in solution culture with the South Carolina cotton fungus, with the South Carolina tobacco fungus, and with the Kentucky 627 tobacco fungus showed external symptoms of wilt in 97.8, 88.9, and 78.7 per cent of the plants, respectively, (Table 4). Much smaller percentages of the plants of resistant

TABLE 4.—*Inoculations of 3 varieties of tobacco in solution cultures with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Tobacco, Burley 31				
S. C. cotton fungus	48	16.7	33.3	25.0
S. C. tobacco fungus	53	3.8	5.7	18.9
Ky. 627 tobacco fungus	48	12.5	12.5	6.2
Uninoculated check	24	8.3	8.3	12.5
Tobacco, Burley 5				
S. C. cotton fungus	45	97.8	100.0	88.9
S. C. tobacco fungus	45	88.9	93.3	66.7
Ky. 627 tobacco fungus	47	78.7	85.1	42.5
Uninoculated check	23	13.0	13.0	8.7
Tobacco, Gold Dollar				
S. C. cotton fungus	46	13.0	23.9	63.0
S. C. tobacco fungus	46	0.0	21.7	56.5
Ky. 627 tobacco fungus	48	6.3	6.3	29.1
Uninoculated check	24	16.7	16.7	16.7

varieties showed external symptoms of wilt, though the fungus was recovered from 56.5 per cent of Gold Dollar plants inoculated with the South Carolina tobacco fungus.

Data obtained from a similar series of inoculations in soil are given in table 5. Plants of Burley 5 were the only ones to show any external symptoms of wilt, though the fungus was isolated from 37.1 per cent of the Gold Dollar plants inoculated with the South Carolina tobacco fungus. The first

external symptoms of wilt in Burley 5 plants appeared 19 days after transplanting to the soil, while, in the culture solution, only 10 days elapsed after inoculation before wilt symptoms were noted. There was more rapid wilting and a considerably higher percentage of infection in the solution cultures than in the soil.

It is evident that wilt in a susceptible Burley tobacco can be produced by inoculations with the Fusaria causing wilt in cotton and in tobacco. The percentages of Burley 5 plants showing external symptoms of wilt with the Kentucky 627 tobacco fungus were less in both soil and solution than with the other isolates. It is probably incorrect to conclude, however, that the Kentucky isolate is less pathogenic than the others, since these percentages

TABLE 5.—*Inoculations of 3 varieties of tobacco in soil with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Tobacco, Burley 31				
S. C. cotton fungus	30	0.0	0.0	3.3
S. C. tobacco fungus	27	0.0	3.7	11.1
Ky. 627 tobacco fungus	35	0.0	0.0	17.1
Uninoculated check	16	0.0	0.0	0.0
Tobacco Burley 5				
S. C. cotton fungus	30	53.3	53.3	70.0
S. C. tobacco fungus	29	58.6	58.6	58.6
Ky. 627 tobacco fungus	31	38.7	41.9	71.0
Uninoculated check	15	0.0	0.0	0.0
Tobacco, Gold Dollar				
S. C. cotton fungus	30	0.0	0.0	23.3
S. C. tobacco fungus	35	0.0	2.9	37.1
Ky. 627 tobacco fungus	32	0.0	0.0	18.8
Uninoculated check	16	0.0	0.0	0.0

are based on a small number of plants. The data of tables 1 and 3 showing the results of the inoculation of a larger number of cotton plants do not indicate such differences. Several months after these experiments were completed, 3 cultures of tobacco-wilt Fusaria from Maryland were obtained. The most virulent culture, "Mudd," was used to inoculate 79 plants of Farm Relief cotton, but only 2 plants showed any symptoms of wilt (Table 6, exp. 1). Since the seed might carry the fungus internally to an extent considerably greater than this, it appeared that the Maryland strain of the fungus was distinctly different from the strains obtained in South Carolina and Kentucky. Accordingly, the 3 Maryland isolates were used to inoculate a susceptible variety of cotton and the Burley 5 and Gold Dollar varieties of tobacco. None of the cotton plants showed any external or internal symptoms of wilt in this test (Table 6, exp. 2), but both varieties of tobacco proved susceptible to all the isolates. Gold Dollar had proved resistant to the South Carolina and Kentucky isolates, but showed 33.3 per cent external wilt with

TABLE 6.—*Inoculations of cotton and tobacco in solution cultures with 3 isolates of Maryland tobacco-wilt Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
Maryland, "Mudd" (exp. 1)	79	2.5	2.5	11.4
Maryland, "Mudd" (exp. 2)	52	0.0	0.0	3.8
Maryland 18 (exp. 2)	62	0.0	0.0	3.2
Maryland 21 (exp. 2)	57	0.0	0.0	3.5
Uninoculated check (exp. 1)	69	0.0	0.0	1.4
Uninoculated check (exp. 2)	43	0.0	0.0	0.0
Tobacco, Burley 5				
Maryland, "Mudd"	67	97.0	97.0	97.0
Maryland 18	62	93.5	95.2	98.4
Maryland 21	63	96.8	98.4	100.0
Uninoculated check	34	0.0	17.6	17.6
Tobacco, Gold Dollar				
Maryland, "Mudd"	57	77.2	94.7	96.5
Maryland 18	54	53.7	94.4	92.6
Maryland 21	54	33.3	74.1	77.8
Uninoculated check	27	0.0	0.0	14.8

the least virulent Maryland isolate and 77.2 per cent wilt with the most virulent isolate.

TABLE 7.—*Inoculations of cotton, cowpea, and Cassia tora in solution cultures with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
S. C. cotton fungus	165	100.0	96.4	93.3
Cowpea fungus	158	0.63	1.3	7.0
Cassia fungus	143	100.0	95.8	96.5
Uninoculated check	74	0.0	4.1[a]	1.3
Cowpea, Cal. Blackeye				
S. C. cotton fungus	153	0.0	0.65	6.5
Cowpea fungus	141	100.0	97.2	95.7
Cassia fungus	152	0.66	2.0	8.6
Uninoculated check	78	0.0	0.0	2.6
Cassia tora				
S. C. cotton fungus	225	0.0	8.4	6.2
Cowpea fungus	207	0.48	1.4	8.2
Cassia fungus	225	0.0	4.0	12.4
Uninoculated check	110	0.0	0.0	0.0

[a] 3 plants with faint, darkened streaks.

Cross Inoculations of Cowpea, Cassia tora, and Cotton

The susceptible Farm Relief 2 cotton, the susceptible California blackeye cowpea, and an apparently highly resistant *Cassia tora* were inoculated in solution culture with wilt Fusaria from each of the host plants.

The data of table 7 indicate that the cowpea organism is quite distinct, and that the organisms from cotton and *Cassia tora* are the same. The few recoveries of Fusaria from cowpeas inoculated with the isolates from cotton and Cassia may have been due to accidental infections or the presence of the wilt fungus in or on the seed, since Kendrick (11) has shown that the fungus can be carried on the cowpea seed. It is possible also that the fungus may grow to some extent in old cortex, out of reach of surface sterilization. Smith (14) made numerous attempts to infect cotton, cowpeas, and watermelons with the wilt Fusaria from these hosts, but, except where the fungus was obtained from its specific host, no infection occurred. Johnson (10) failed to obtain infection of cowpeas from inoculations with a Maryland tobacco-wilt fungus. Carpenter (5) obtained no infection of Brabham cowpeas with an okra Fusarium.

Inoculations of Cotton, Okra, and Tobacco

Carpenter (5) inoculated 99 okra plants with the cotton-wilt fungus in 3 tests, and obtained only 1 wilted plant. He did, however, get 87 per cent wilt from the inoculation of 43 cotton plants with the okra fungus. Fahmy (9) failed to obtain infection of okra with the Egyptian cotton-wilt fungus.

The data of table 8 show clearly that the okra fungus will cause wilting

TABLE 8.—*Inoculations of okra, tobacco, and cotton in solution cultures with wilt Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Okra, Clemson Spineless				
Okra fungus (exp. 1)	80	91.2	100.0	93.8
Okra fungus (exp. 2)	84	100.0	100.0	83.3
S. C. cotton fungus	66	98.5	98.5	86.4
Maryland tobacco fungus "Mudd"	74	0.0	1.4	4.0
Uninoculated check (exp. 1)	41	0.0	0.0	0.0
Uninoculated check (exp. 2)	56	0.0	8.9	7.1
Tobacco, Burley 5				
Okra fungus	23	100.0	100.0	78.3
Uninoculated check	27	0.0	0.0	3.7
Cotton, Farm Relief 2				
Okra fungus	90	58.9	68.9	62.2
Uninoculated check	80	0.0	0.0	1.2

of okra, a susceptible Burley tobacco, and a susceptible cotton. The cotton fungus also caused wilting of okra. The "Mudd" isolate of the Maryland tobacco fungus, however, did not produce any visible wilt of okra.

Inoculations of Okra, Tomato, Cotton, and Watermelon

The data of table 9 show that the tomato was not attacked by the wilt

TABLE 9.—*Inoculations of okra, tomato, cotton, and watermelon in solution cultures with wilt Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Tomato, Improved Earliana				
Tomato fungus	95	94.7	100.0	98.9
Okra fungus	91	0.0	1.1	5.5
Watermelon fungus	101	0.0	0.0	5.9
Uninoculated check	69	0.0	0.0	2.9
Watermelon, Florida Giant				
Watermelon fungus	49	100.0	100.0	95.9
Tomato fungus	45	0.0	0.0	2.2
S. C. cotton fungus	88	0.0	0.0	0.0
Uninoculated check	34	0.0	0.0	0.0
Okra, Clemson Spineless				
Tomato fungus	65	0.0	0.0	3.1
Uninoculated check	56	0.0	8.9	7.1
Cotton, Farm Relief 2				
Tomato fungus	59	0.0	0.0	0.0
Uninoculated check	43	0.0	0.0	0.0

fungi from okra or watermelon. Since the data from other experiments indicate that the wilt Fusaria from cotton, okra, Cassia, South Carolina tobacco, and Kentucky tobacco are the same, it seems reasonable to conclude that the tomato is not subject to wilt by any of these fungi. The tomato-wilt fungus[8] also did not produce wilt in watermelon, okra, or cotton. Bohn and Tucker (4) have indicated that the parasitism of *Fusarium lycopersici* is limited to the genus *Lycopersicon*.

The cotton fungus did not cause wilt of the watermelon, which agrees with the results obtained by Smith (14) many years ago.

DISCUSSION

The writers' experience with Fusaria has been confined largely to the section Elegans. It is generally recognized that the Fusaria may vary widely in the morphological and physiological characters, including patho-

[8] Culture supplied by F. L. Wellman.

genicity, that are utilized in taxonomy. No attempt will be made to review the extensive literature on this subject, and only those references that bear directly on the present investigation will be cited.

In earlier studies of cotton wilt by the senior writer, a large number of isolations of the fungus were made from wilting cotton stalks, chiefly from various sections of South Carolina. After these isolates had been in culture for different lengths of time, 19 cultures representing the range of cultural characteristics were selected for further study. When single-spore cultures were made of some of the isolates differing most in cultural characteristics. great variability was revealed (2). Variants appeared as early as the second transfer showed a tendency to dominate the parent culture. A series of variants, quite similar to the range of those reported for tomato wilt by Wellman and Blaisdell (18), were obtained. Some of the cotton cultures could not be identified as *Fusarium vasinfectum* according to descriptions given by Wollenweber and Reinking (19), yet they caused cotton wilt. Johnson (10) made cultural comparisons of isolates of *F. oxysporum* from potatoes with his fusarium cultures from tobacco and mentions the difficulties in making a decision as to their identity, since some of the potato cultures were "sub-normal," one in particular being less virulent than the vigorous mycelial types from tobacco. Only the vigorous mycelial types have been used for the inoculations reported in this paper, but one of the "sub-normal" types of the cotton-wilt fungus used in a previous study (2) proved as virulent as the vigorous mycelial types. Other "subnormal" or appressed cultures, however, were less virulent than most of the vigorous mycelial types, which agrees with the results of others studying several wilt Fusaria.

During the period from 1932 to 1935, inclusive, 4 attempts were made to differentiate culturally, 8 single-spore isolates of *Fusarium vasinfectum* by the technique of Coons and Strong (6), using the toxic malachite-green and crystal-violet dyes. Fairly distinct differences among several of the isolates were apparent in a single test, but repetitions of the experiment produced results inconsistent with those of the other tests. Since it has been found (2) that variants of *F. vasinfectum* may occur after 1 or 2 transfers, and the cultures used in the dye tests were transferred from 1 to several times between the tests, it seems reasonable to assume that variant forms may have been used in the later tests, which reacted differently to the toxic dyes than did the original cultures. Padwick (13) states, "A number of isolates of *F. udum* were compared with *F. lateritium* var. *uncinatum* Wr. and also with cultures of all varieties and physiologic forms of *F. vasinfectum,* obtained from the centraalbureau voor Schimmel cultures, Baarn. This experiment strikingly confirmed the previous conclusion that *F. udum* is a separate species from *F. vasinfectum.*" The writers are not familiar with the 8 cultures of *F. vasinfectum* examined by Padwick, but one wonders what might have been the conclusion if the wide range of forms they have had in culture had been included in his study. Padwick was not able, however, to distinguish by morphological and cultural studies between the isolates of

F. udum that cause wilt only of pigeon-pea and those that cause wilt only of sun-hemp.

Snyder and Hansen (16) have obtained types of the watermelon wilt Fusarium that fall into sub-sections Orthocera, Oxysporum, and Constrictum of the section Elegans, as proposed by Wollenweber and Reinking (19). It was proposed by Snyder and Hansen (16) that the section Elegans be converted into 1 species, *F. oxysporum*, with a number of biologic forms recognized principally by their selective pathogenicities. According to this scheme, *F. vasinfectum* Atk. becomes *F. oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen, and *F. oxysporum* Schl. v. *nicotianae* Johns. becomes *F. oxysporum* f. *nicotianae* (Johns.) Snyder and Hansen.

When it was discovered that the Fusaria from Burley tobacco collected in South Carolina and Kentucky would also cause cotton wilt, and that the cotton wilt Fusarium would cause wilt of Burley tobacco (1), it was assumed that both names of the fungi were unnecessary. With the later discovery that the Maryland tobacco-wilt organism would not infect cotton and okra, it became clear that both names might be needed. The Maryland tobacco-wilt organism is also different from the other two tobacco organisms in that it causes wilt in the flue-cured variety, Gold Dollar, where the others do not. If one were comparing the several tobacco organisms by inoculating the susceptible Burley variety, the conclusion would be that they are the same. If the flue-cured variety were added to the tests, the different pathogenic potentialities could be indicated by designating them as different physiologic races. If cotton be added to the test, the situation becomes slightly more complex, since the Kentucky and South Carolina isolates also cause cotton wilt. On the basis of priority, the 2 latter isolates, according to the classification of Wollenweber and Reinking (19), should be *F. vasinfectum*. When increasing the host list of *F. vasinfectum* to include *Cassia tora* and tobacco, it would be necessary to state that this organism causes tobacco wilt in only certain types of tobacco (Burley strains). If the classification of Snyder and Hansen (16) be used, the name would be given as *F. oxysporum* f. *vasinfectum* with the increase in host list as suggested above. The Maryland isolate which affects Maryland Broadleaf and flue-cured types would retain the name of the tobacco organism as originally described.

Three series of cross inoculations involving the sweet potato have been made but no detailed data are presented at this time, since the results in general are rather uncertain. The South Carolina and Kentucky tobacco-wilt fungi have not produced distinct external symptoms of wilt in the sweet potato in all tests, though Smith and Shaw (15) have reported that the tobacco-wilt fungus with which they are working does cause wilting of the sweet potato.

Pathogenicity tests with the wilt-producing Fusaria are not short and easy procedures, but it appears that many cross-inoculation experiments are necessary before the pathogenic potentialities of this group will be known. The morphological and cultural studies thus far recorded fall far short of giving a satisfactory method for identifying many of these organisms.

SUMMARY

Inoculations with the wilt Fusaria from *Cassia tora*, cotton, tobacco, okra, tomato, watermelon, and cowpea have been made chiefly by means of a solution-culture technique, though some soil inoculations also were employed.

The isolates from cotton, okra, Cassia, and tobacco from South Carolina and Kentucky caused wilt with these various hosts; thus it appears that one fungus is involved. A susceptible Burley tobacco was attacked, but a flue-cured variety, Gold Dollar, showed little or no wilt when inoculated.

Tobacco isolates from Maryland, however, caused wilting of both the Burley and the flue-cured variety of tobacco, but not of cotton and okra.

The weed, *Cassia tora*, found growing in cotton fields was attacked by the cotton-wilt fungus. The tomato, watermelon, and cowpea fungi are probably pathogenic only to their respective hosts or closely related hosts, since the tomato fungus did not cause wilt of watermelon, okra, or cotton, the watermelon fungus did not cause wilt of tomato, and the cowpea fungus attacked neither cotton nor Cassia.

The following is a brief summary of the results of the inoculations.

Fusarium from	Caused wilt of	Caused no wilt of
Cotton	Cotton, Burley 5 tobacco, okra, Cassia	Gold Dollar flue-cured tobacco, watermelon
South Carolina tobacco	Cotton, Cassia, Burley 5 tobacco	Gold Dollar flue-cured tobacco
Kentucky tobacco	Cotton, Burley 5 tobacco	Gold Dollar flue-cured tobacco
Okra	Okra, Burley 5 tobacco, cotton	Tomato
Cassia tora	Cassia, Burley 5 tobacco, cotton	
Maryland tobacco	Burley 5 tobacco, Gold Dollar flue-cured tobacco	Cotton, okra
Tomato	Tomato	Watermelon, okra, cotton
Watermelon	Watermelon	Tomato
Cowpea	Cowpea	Cotton, Cassia

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THE EFFECT OF LIGHT AND TEMPERATURE ON THE VIABILITY OF UREDIOSPORES OF CERTAIN CEREAL RUSTS[1]

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INTRODUCTION

It has been shown that urediospores and aeciospores of some of the cereal rusts, notably *Puccinia graminis* Pers., may be carried long distances by the wind. According to Stakman (18), there is conclusive evidence that urediospores of *P. graminis* are sometimes blown northward from Texas, Oklahoma, or Kansas into the spring-wheat States in such numbers as to be responsible for the initiation of rust epidemics.

Urediospores of *Puccinia graminis* from a distant source often are caught near St. Paul, Minnesota, in large numbers on spore traps in June; and in July the numbers are sometimes almost inconceivably large. At this time, from a few hundred to a few hundred thousand urediospores may be deposited per square foot at the level of growing grain plants within 24 hours during so-called spore showers. These spores are blown into the spring-wheat area by south winds and very often during periods of rather warm, bright, dry weather, when there is insufficient moisture for them to germinate. It is well known that high temperatures and light are likely to affect adversely the development of the cereal rusts (1, 4, 7, 8, 9, 10, 12, 13, 14, 20, 22). These factors also affect the viability of spores, but to what degree has not been known very precisely.

As information regarding the number of spores caught in spore traps has been used as one basis for attempting to determine whether epidemics are likely to occur, it seemed desirable to get as much information as possible regarding the longevity of the spores on the plants with a view to establishing if possible a correction factor based on the amount of sunshine and the temperature and the time elapsing between the spore shower and the first dew or rain.

For this reason the writer attempted to determine the effect of sunlight and temperature on urediospores of *Puccinia graminis tritici* Eriks. and Henn., *P. coronata* Corda, *P. rubigo-vera tritici* (Eriks. and Henn.) Carl., *P. graminis avenae* Eriks. and Henn., and *P. graminis secalis* Eriks. and Henn. As races of a given species conceivably could differ in their resistance to light and high temperature, several physiologic races of *P. graminis*

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tritici and *P. graminis avenae*, as well as *P. coronata*, were included in many of the studies. There was no attempt to study relative humidity or any of the other factors that have been reported (5, 7, 15, 21) as reducing spore viability.

MATERIALS AND METHODS

Spores of *Puccinia coronata*, *P. graminis tritici*, and *P. rubigo-vera tritici* for the preliminary experiments were obtained from two different sources, the fields of University Farm, St. Paul, Minn., and plants grown in the greenhouse.

Rust collections used for the subsequent experiments were obtained from the Federal Rust Laboratory, University Farm, St. Paul, and propagated on seedlings in the greenhouse. The usual methods of inoculation and incubation were used (17) for races 11, 36, 38, and 56 of *P. graminis tritici*, races 2 and 6 of *P. graminis secalis*, races 1 and 45 of *P. coronata*, and *P. rubigo-vera tritici*. As soon as uredia were well formed, mature spores were collected by gently shaking the infected plants over a smooth funnel leading to a glass vial. The conditions for spore production among different rusts were almost identical, unless specific treatments were given for certain purposes.

Methods of Exposure

Immediately after the spores were collected from the greenhouse, they were put on a piece of white paper over a piece of filter paper in Petri dishes. The dry spores were evenly distributed before exposure to the direct sunlight, because Fulton and Coblentz (3) pointed out that the outer spores of clumps have a protective shading effect on those in the center of the mass, and the latter survive exposures several times longer than is required to kill single detached spores. Usually, the dishes were covered with white cellophane and checks were covered with black tar paper, although no covers were used in the preliminary experiments. When testing the varying qualities of daylight, blue cellophane and red Du Pont cellophane, with known percentages of light transmission, were used as covers.

These Petri dishes of dry spores were exposed continuously outdoors; but, after a certain specified number of hours, they were carried back to the laboratory and a small amount of each sample was removed with sterile forceps and placed in separate, clean Syracuse dishes. The Petri dishes were again put outdoors for further exposure and later samplings.

The records for temperature and light intensity were usually taken 3 times a day. Thermometers were inserted in Petri dishes covered as for spore exposures. The instrument used to measure light intensity in the preliminary experiments consisted of a glass bulb fitted with a thermopile, and the record was in terms of g. cal./min./cm.^{2} For the succeeding experiments, a Weston Illumination meter, model 603, was used and light intensity was recorded in foot-candles. According to Duggar (2) Kimball gave an average value of 6700 foot-candles for 1 g. cal./min./cm.^{2} The

former records, therefore, were converted into foot-candles based on Kimball's method.

The initial germination in a spore collection varied from time to time and also with the different rust species used. Usually it was between 70 and 100 per cent, but occasionally for certain spore collections, it fell to 50 or even lower. In all cases in order to have the rates of decline in viability more comparable, the initial germination percentage for every spore lot was considered as 100. Subsequent germination of each spore lot after exposure was figured as a proportion of this base.

Methods for Spore Germination

After a certain number of hours' exposure, spores were distributed in Syracuse dishes for germination tests. Distilled water was added to the dishes, which were then left for 3 to 10 hours at room temperature (20–25° C.) before spores were counted. Temperatures were relatively uniform, for when spores of different races of certain species of rusts were tested for germination they were always kept side by side. Approximately 10 cc. of water, distilled in a metal still, were used for each Syracuse dish. The total number of spores counted in each dish was from 300 to 500. Usually, at least 3 tests were made for each species, variety, or race of rust in each experiment, but only representative data are presented here.

THE GERMINATION OF UREDIOSPORES FROM DIFFERENT SOURCES

Urediospores produced in the greenhouse were compared with those from the field as to their ability to withstand exposure to air and light, for it was realized that the source of spores, especially during the winter, might be an important factor in experiments on viability. Mature urediospores of *Puccinia graminis tritici*, *P. coronata*, and *P. rubigo-vera tritici* from the two sources (greenhouse and field) were exposed continuously outdoors during July, 1937, and their germinability tested after intervals varying from 2 to 125 hours. There was no evidence of marked differences in viability of the urediospores from the two different sources, so that for the later experiments urediospores produced in the greenhouse were used. This practice enabled one to use pure races of rusts, to have material available in all months of the year, and to control to a slight extent the conditions for urediospore production.

COVERING MATERIALS FOR PETRI DISHES AND THEIR EFFECT ON LOSS OF UREDIOSPORE VIABILITY

Since exposure in uncovered dishes was impractical for the outdoor experiments whenever large numbers of spores were in the air, when strong winds or storms prevailed, or when snow or rain fell, various covering materials were used, including white cellophane, glass, a double layer of cheesecloth, and black tar paper. The cellophane interfered very little with passage of light waves, but, in windy or stormy weather, it split easily.

Cheesecloth reduced light intensity only slightly, but did not prevent disturbance and loss of the exposed spores. The black paper reduced the light greatly and the temperatures under such covers were apt to be higher than those under glass or cellophane covers. The results of numerous experiments, however, show clearly that, provided temperatures are not extremely high, viability of urediospores is retained much longer under a black cover that reduces light than under a cover that transmits most of the light reaching it. In table 1 are given figures for the germinability of urediospores of race 11 of *Puccinia graminis tritici*, which may be taken as representative of the other rust races and varieties studied. *P. rubigo-vera tritici*, *P. coronata*, and races 36, 38, and 56 of *P. graminis tritici* were included in the experiments, and the results for all were very similar in each experiment.

Obviously, there were physical difficulties in starting all the exposures, the replicates, and all the different race collections at the same time, but care was exercised in observing, according to the starting sequences. Insofar as possible, exposures were begun between 7:30 and 9:00 A.M. Length of exposure is recorded in hours, but, since sunlight was the important factor being investigated, the number of hours of sunlight during exposure usually has been given as well. Petri plates exposed more than 8 hours were left out continuously day and night, and the total time of exposure was 2, 3, or more times greater than the hours of daylight recorded.

With exposure to daylight under a cellophane, a glass, or cheesecloth cover viability decreased rapidly at low and moderate temperatures, more rapidly than with the black paper cover (Table 1). As long as temperatures remained moderate, such protection from light by the black tar paper cover provided for retention of viability for 4 to 8 days or even 24 to 28 days longer than did a transparent cover.

TABLE 1.—*The percentage germination of urediospores of Puccinia graminis tritici race 11 after exposure, under different covers, to daylight and to various temperatures*

Exposure in hours[a]	−10° to +6° C.			12° to 26° C.				27° to 42° C.	
	Low light		Black paper	Low light		Black paper	High light	High light	Black paper
	Cloth	Glass		Cloth	Cellophane		Cellophane	Cellophane	
0	100	100	100	100	100	100	100	100	100
8 (8)	2	2	24	61	41	69	55	20	tr
15 (8)	.....	.....	.....	.....	.....	.....	29	3	0
30 (15)	1	2	6	16	14	27	1	0	0
50 (20)	3	1	5	11	12	18	tr	.....	.....
100 (40)	1	1	5	14	7	10	.....	.....	.....
150 (60)	0	0	4	6	7	5	.....	.....	.....
400 (153)	.....	.....	5	1	1	4	.....	.....	.....
460 (175)	.....	.....	5	0	tr	1	.....	.....	.....
500 (190)	.....	.....	5	0	0	1	.....	.....	.....
600 (225)	.....	.....	2	.....	.....	tr	.....	.....	.....
800 (300)	.....	.....	1	.....	.....	0	.....	.....	.....
1000 (400)	.....	.....	tr	.....	.....	.....	.....	.....	.....

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period.

Whenever temperatures were high, then temperature assumed a primary role and daylight became an accessory factor in loss of urediospore viability (Table 1). In nature, however, it is only occasionally that temperature rises so high as to become a primary factor for urediospore viability of the rusts considered in this paper. In nature it is possible that the cereal foliage reduces sunlight intensity, and temperature as well, and prolongs somewhat the germinability of urediospores.

THE EFFECT OF HIGH TEMPERATURES ON THE VIABILITY OF UREDIOSPORES OF SEVERAL CEREAL RUSTS

Since there was evidence that spores were killed by high temperature, as well as by light, the germination of spores kept in incubators at 44 ±, 50 ±, and 60 ± ° C., respectively, was studied. Three experiments with 3 replications for each rust were made at different dates. Mature spores of the 4 races of *Puccinia graminis tritici,* the 2 of *P. graminis avenae, P. graminis secalis, P. rubigo-vera tritici,* and the 2 races of *P. coronata* were exposed.

Data for a representative sample, race 36 of *Puccinia graminis tritici,* are presented in table 2. Urediospores withstood a temperature of 44° C. very well for 2 days; and, even after 60 hours, 8 per cent of the spores germinated.

At 50° C. viability was lost more rapidly, so that, after 2 days, only 1 per cent of the spores germinated, and within 60 hours all were dead.

Exposure to 60° C. brings about a still more rapid decline in germinability. Within 4 hours, more than half the spores lost their viability, and at the end of 10 hours less than 10 per cent were able to germinate. Nearly all spores were dead after 15 hours at 60° C.

TABLE 2.—*The effect of high temperature on the viability of urediospores of Puccinia graminis tritici 36*

Exposure in hours	Average percentage[a] germination of spores after exposure at		
	44° C.	50° C.	60° C.
0	100	100	100
8	95	45	26
10	75	41	8
15	79	23	tr
20	67	11	0
25	52	12	0
36	40	4	0
48	28	1	0
60	8	0	0

[a] Each figure is the average of three replicates.

Attempts were made to harden certain spore lots by exposure for seven hours at room temperature before the exposure to 60° C. Such treatment, however, did not enable the spores to withstand the high temperature any better than did nontreated spores.

In nature we may safely say that such high temperatures never obtain

in the Upper Mississippi Valley, although 40° C. and slightly higher have been recorded on occasion in Minnesota. Intense sunlight in nature, however, undoubtedly adds its inimical effects to those of high temperature, and the viability of urediospores in the air is reduced more rapidly than when either factor acts alone.

LIGHT INTENSITY AND LOSS OF UREDIOSPORE VIABILITY

It is difficult to separate the factors of light intensity and temperature when studying retention of viability in rust urediospores, but two series of experiments are available in which the predominant range of temperature was 14 to 24° C. in one instance and 16 to 24° C. in the other, while the light intensity was held to a low limit in one but soared to 7,000 foot-candles in the other. Data for the germinability of races 36, 38, and 56 of *Puccinia graminis tritici* under these two sets of conditions are in table 3.

One criticism of the work might be that the experiments occurred in two different years. Nevertheless both lots were collected from the greenhouse at about the same time of year (within 7 weeks), and the initial germination percentages were very similar in both series. With the practice of setting the initial germination count at 100 per cent, the comparison should be a very good one.

A direct correlation between the lower light intensity and retention of viability is evident. During the first 3 days of Experiment II the average light intensity was rather low, and it was not until the fourth day that light intensity increased enough to differentiate the data of Experiment II from those of Experiment I.

Germination counts for *Puccinia graminis tritici* race 11, for *P. coronata*

TABLE 3.—*The effect of light intensity on loss of viability, as measured by percentage germination, in urediospores of races 36, 38, and 56 of Puccinia graminis tritici*

Exposure[a] in hours	500–1500 foot-candles			1000–7000 foot-candles		
	Race 36	Race 38	Race 56	Race 36	Race 38	Race 56
0	100	100	100	100	100	100
8 (8)	87	82	78	96	100	85
25 (10)	.....	.....	.....	70	78	69
50 (20)	.....	.....	.....	58	72	72
80 (36)	18	50	39	58	15	38
123 (48)	16	40	34	42	26	41
150 (60)	.....	.....	.....	27	20	26
175 (75)	15	37	33	13	10	28
225 (100)	14	28	33	.....	.....	.....
300 (140)	9	24	29	0	0	tr
365 (175)	8	25	30	.....	.....	.....
420 (200)	3	21	28	0	0	0
570 (270)	1	15	14	.....	.....	.....
650 (300)	1	2	2	.....	.....	.....
750 (350)	0	1	tr	.....	.....	.....
840 (430)	0	tr	tr	.....	.....	.....

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period. The temperature ranged from 14 to 24° C.

races 1 and 45, *P. graminis avenae* races 2 and 6, and *P. graminis secalis* are very similar to those presented and would have illustrated the effect of light intensity as well as the data chosen for table 3.

At low light intensities more than 10 per cent of the spores remained viable after exposure to sunlight for 270 hours, except for race 36 of *Puccinia graminis tritici* whose viability dropped more rapidly. The 270 hours of sunlight at the time of the experiments in February and March means about 23 to 24 days of exposure, a relatively long period for urediospore survival in the air.

When light intensity reached a daily maximum of 7,000 foot-candles, at least 10 per cent of the urediospores retained the power to germinate after a 75-hour exposure, but none survived 270 hours of sunlight. With higher light intensities, which sometimes surpass 10,000 foot-candles on a June day in Minnesota, the survival time for urediospores may be even shorter.

QUALITY OF LIGHT AS IT AFFECTS UREDIOSPORE VIABILITY

The short waves of the ultra-violet and the blue parts of the spectrum generally have been more effective in decreasing germinability of fungus spores than have the longer waves (2, 3).

Cellophane Filters

In order to determine the relative effects of different qualities of direct sunlight, filters of 3 colors of Du Pont cellophane, with known percentages of transmission, were used to cover the exposure dishes. The colorless, red, and blue filters were compared with black paper covers for their effects on germinability of urediospores of all the rust species, varieties, and races used in other experiments.

All spores for these experiments were collected during October through December in 1938 or in 1939, and the initial germination counts for all spore lots were usually rather low. As in all other experiments, the germination counts after various exposure periods represent percentages of the initial germination counts before exposure.

From table 4 it is evident that urediospore viability declines more rapidly under the colorless cellophane filter than under the blue and red cellophanes or the black paper covers. The data presented for race 2 of *Puccinia graminis avenae* are representative of the other races, varieties, and species of rusts used in 8 different series of experiments, except *P. graminis secalis*. For this variety the loss of viability was about equal under all covers, and it is possible that its spores are slightly more resistant to sunlight than those of other varieties and species.

Ultra-violet Light

In October and November, 1938, mature urediospores were irradiated with ultra-violet, and their germination tested after various intervals. The

spores were evenly distributed in Syracuse dishes within Petri dishes, which were left open for irradiation or were covered with colorless cellophane. A slanted board, 32 inches from the light source, supported the dishes for irradiation. The Eveready Therapeutic "C" carbon arc furnished wave lengths of 2,700–3,000 Ångstrom units (see 11), and the temperature at the irradiation distance was between 30 and 36° C., while measurable light intensity was about 800 foot-candles. Spores were irradiated 15 or 20 minutes, then tested for germination in distilled water at room temperature.

TABLE 4.—*The percentage germination of urediospores of Puccinia graminis avenae race 2 exposed under covers of colored cellophane to light of different quality*

Exposure[a] in hours	Colorless	Blue	Red	Black
0	100	100	100	100
8 (8)	48	75	75	59
25 (10)	23	15	15	16
32 (15)	7	7	10	8
54 (20)	3	3	10	3
98 (35)	2	10	14	13
144 (48)	0	9	13	13
215 (72)	0	3	9	9
290 (96)	0	1	5	4

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period. Sunlight intensity was approximately 3500 to 6000 foot-candles and temperature was 12–27° C.

There is a general tendency toward reduction in germination after exposure for 15 minutes to ultra-violet light (Table 5), yet a surprisingly large number of spores were not killed by the 20-minute exposure.

Urediospores of *Puccinia graminis avenae* seemed to be more sensitive to ultra-violet than those of the *secalis* and *tritici* varieties of *P. graminis* or those of other rust species. Direct irradiation was often slightly more injurious than irradiation through cellophane, but it was not consistently so.

In each case one series of figures was obtained for spores collected and stored for a week at 10° C. before exposure to ultra-violet and another series of figures for spores collected and exposed immediately. Storage of spores sometimes seemed to confer a very slight resistance to ultra-violet, but the effects were inconsistent.

CONDITIONS OF SPORE PRODUCTION THAT MIGHT AFFECT UREDIOSPORE VIABILITY

Time of Formation in the Uredium

A single uredium in nature produces urediospores continuously for many days or sometimes even for weeks before the uredium changes to a telium or before it dries and stops drawing nutriment from the host. Stakman and Levine (19) showed that there was no apparent difference in the degree of infection produced by rust spores of different ages, but it was not known

whether urediospores, formed early in the life of the uredium, would be more or less resistant to the effect of sunlight than those formed late in the life of the uredium, when supplies of the host nutriment might be more or less depleted.

Early crops of mature spores were collected soon after uredia had formed, usually 12 or 13 days after inoculation. Seven or 8 days later another crop of mature spores was collected from the same uredia. In some of the experiments, by storing the first spore collections at 10° C. for a week, it was possible to expose both collections to the same light and temperature and compare their relative sensitivities. According to the reports of Hoerner (6), Peltier (13), Schilcher (16), and Rosen and Weetman (15), short storage does not reduce the vitality of spores to any considerable extent.

TABLE 5.—*The percentage germination*[a] *of urediospores of various species of Puccinia irradiated with ultra-violet light directly or through cellophane covers*

Rust urediospores	Irradiation, 15 min.		Irradiation, 20 min.	
	Cover	No cover	Cover	No cover
P. gr. avenae 2	22	18	13	15
do 6	7	7	7	6
P. gr. secalis	80	76	65	69
P. gr. tritici 11	82	75	75	72
do 36	62	60	58	52
do 38	54	41	50	43
do 56	57	55	40	33
P. coronata 1	85	78	81	75
do 45	78	66	52	49
P. rubigo-vera tritici	75	65	71	64

[a] The percentage germination without irradiation was arbitrarily placed at 100.

In none of the experiments were there evident any consistent differences in sensitivity to light in the two spore crops. Loss of viability proceeded at the same rate in both collections when the exposures to light were comparable. When the two spore crops were subjected to 50° or 60° C., the spores of the later crop sometimes withstood extreme temperatures for a few hours longer than spores of the early crop. At moderate temperatures, however, no differences were evident. Apparently the spores formed early in the uredium mature rapidly and are as vigorous as those formed a week later, when the uredium usually is considered in its prime. Whether still later crops of urediospores would have a lower vitality or a greater resistance to high temperatures and drying is still a question.

Light Condition During Uredial Development

Throughout the years during which the present work was done, there were general observations on the relative initial vigor of spore lots collected at different seasons or under different environmental conditions. Indica-

tions are that urediospores formed during long-day seasons, when sunshine is prevalent and relatively bright, are more vigorous as measured by a higher initial germinability than are urediospores formed during a short-day winter season, when cloudy days prevail. With nearly all of the rusts studied here, urediospores formed in the greenhouse during November or December germinated in fewer numbers (usually from 35 to 70 per cent) when freshly collected than did urediospores formed in any other month of the year (usually 75 to 95 per cent). The single possible exception seemed to be *Puccinia graminis secalis,* a variety in which initial germination of urediospores formed in November or December sometimes was remarkably high (86 per cent).

To determine whether a greater than usual reduction in light during November and December would result in more pronounced weakening of urediospores formed in those months, half-shade was provided for inoculated plants by 1 or 2 layers of muslin. Urediospores produced under half-shade were compared with those produced under the full light of November and December, and there were no consistent differences in initial germinability of the freshly collected spores or in the decline in germinability resulting from exposure to sunlight or to high temperatures.

THE EXPOSURE OF RUST SPORES ON CEREAL FOLIAGE AND THE INFECTION OF PLANTS AFTER EXPOSURE

In the normal course of a growing season many of the urediospores, drifting or being blown about by air currents, eventually settle on grain fields and cereal foliage. Environmental conditions are not always favorable for immediate germination and infection, so the deposited spores often are exposed to the elements for several days longer. As yet, no one is certain whether most of the spores retain their ability to infect the cereal host for some time after deposition or whether only a few are able to do so. It is possible that under field conditions the foliage protects urediospores from the full effects of direct sunlight and that it modifies temperature so that many spores survive. The experiments concerning this part of the work were made with seedlings growing in 4-inch pots in the greenhouse. The susceptible wheat variety Marquis and the moderately resistant oat variety Anthony were brushed with dry urediospores of their respective rusts about 5 or 6 days after planting, when the seedlings were 2 to 3½ inches high. Immediately after the spores were brushed on, some of the plants were exposed to direct daylight in the greenhouse, others were exposed to diffuse daylight while in a shaded place in the greenhouse, and a few were put in moist chambers and atomized lightly with water to allow for immediate infection and to serve as checks for the exposed plants. After exposure for a designated length of time the seedlings were placed in a moist chamber, atomized lightly with water, and left for 48 hours.

The success of infection was determined by counting the lesions that developed on plants within 10–12 days after the moist chamber incubation

(Table 6). The number of uredia on a plant was somewhat erratic, which is not surprising when one realizes how difficult it is to brush spores evenly over the leaf surfaces and to avoid washing off spore clumps during the atomizing. Nevertheless, uredia were numerous enough to signify that considerable numbers of spores remained viable and infected the cereals when opportunity arose. Even after exposures of more than 4 days, with about 48 hours of direct light, good rust infection resulted within 10 or 12 days after moisture was supplied for spore germination. With the longer exposures, particularly on wheat, a diffuse light was less harmful to spore viability than was direct light. High temperatures at the beginning of the experiment may have been responsible for the rather low averages for the check plants in the experiments.

We may conclude, however, that many of the urediospores deposited on cereal leaves, especially on the lower shaded foliage that is protected from direct sunlight, may remain viable for at least 4 days and probably longer, there being no necessity for moisture in amounts that would allow for rust infection at the exact time of the spore shower.

TABLE 6.—*The average number of uredia formed on oats and wheat seedlings that were dusted with urediospores of Puccinia coronata, P. graminis tritici 36, of P. rubigo-vera tritici and were exposed to sunlight for various times prior to incubation in moist chambers*

Exposure[a] in hours	*P. coronata*		*P. gr. tritici* 36		*P. rubigo-vera tritici*	
	Full light	Diffuse light	Full light	Diffuse light	Full light	Diffuse light
0	20	20	10	10	18	18
8 (8)	34	29	34	26	61	68
10 (10)	32	28	29	10	40	66
28 (15)	23	22	36	35	48	71
46 (20)	29	41	18	17	25	52
51 (25)	13	27	17	15	5	55
75 (36)	4	2	10	19	3	27
100 (48)	2	6	4	20	2	36

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period. Light intensity was approximately 5000–7500 foot-candles, and temperature was 18–27° C.

DISCUSSION AND CONCLUSIONS

It has been known for some time that urediospores of the cereal rusts can be carried long distances by the wind and that many of the spores are viable after several days in the air. They often survive fairly long journeys in the air and serve as inoculum for cereal fields at some distance from their source. Among the factors that are unfavorable for urediospore survival are high light intensity and high temperature, which are discussed in this paper. It has been shown that urediospores are sensitive to exposure to sunlight, and the present work demonstrates a direct relationship between light intensity and the rapidity with which viability is lost.

On the basis of the present experiments, it is probable that at low light intensity some of the urediospores in the air remain viable for more than a month, but at moderate light intensities, with a daily maximum of 7000 foot-candles, which may be expected in May and early June in Minnesota, most of the spores in the air lose their viability in 12 to 14 days. With greater light intensities it is estimated that the spores survive an even shorter time.

In spite of the seemingly rapid loss of viability by most of the spores when light intensity is high, there would still seem to be a fair margin of safety for spore survival and for effectiveness of inoculum in an ordinary season. The heavy spore showers occur when days are long and bright, but the number of spores is generally so high that a small percentage of viable spores is sufficient to produce good rust infection. Furthermore, spores may be carried at a rapid rate by the prevailing winds, so that 1 to 3 days suffice for transfer of inoculum to new, far-distant, susceptible fields. Furthermore, although bright sunny days are expected in June, there usually are 1 or 2 cloudy days, or days of diffuse light, alternating with 3 to 5 sunny days. It is not until July that we expect long stretches of days with bright sunshine and high temperatures throughout the Upper Mississippi Valley.

High temperatures as well as intense light are detrimental to survival of urediospores. In nature, however, it is doubtful if temperatures ever rise to such heights as were used in the experiments, and if they do occur during the growing season for cereals, seldom last very long.

Survival of urediospores after they are deposited on cereal foliage is another of the important aspects of the rust epidemiology situation. Although urediospores may survive several days in the air, several additional days may elapse after spores are deposited on cereal plants before sufficient moisture becomes available for spore germination and infection. It appears from the present study that deposited spores may be somewhat protected from the adverse effects of light and temperature and that many of them may survive on the dry foliage surfaces for longer than 4 days.

It may be assumed, therefore, that in most seasons large percentages of the urediospores present in the atmosphere at a given time may lose their viability before there is opportunity for them to function as inoculum for new cereal plantings, but that relatively small proportions of the vast numbers of urediospores serve to spread and perpetuate the rust.

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INDEXING CHERRY YELLOWS ON PEACH

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INTRODUCTION

In 1939, Keitt and Clayton (5) reported a bud transmissible chlorosis on Montmorency cherry (*Prunus cerasus* L.) in Wisconsin. This disease, characterized by yellow leaves and premature defoliation, probably had been present in the commercial districts of Wisconsin, Michigan, and New York (8) for at least 20 years prior to this discovery. The delay in identification is understandable when the various agents in the leaf-yellowing complex in sour cherries are considered—sprays, insects, drought, fertilization, fungi, mechanical injuries, and a virus.

Cherry yellows spreads relatively slowly in orchards, probably through the instrumentality of a yet unknown insect vector. Moreover, it is not an aggressive disease (4, 6, 7) and does not kill the trees, but gradually reduces fruiting to a commercially unprofitable level. The yellowing and dropping of the older leaves in early summer each year causes a decline in tree vigor in which the spur system is markedly reduced until fruit bearing becomes confined principally to the previous season's growth (Fig. 1, A). Since the fruit is often larger than normal, and of excellent quality, growers commonly retain such trees, even after they become unprofitable producers.

Cherry yellows has been found in nursery plantings (3). In the nursery, bud sticks for propagation purposes are ordinarily taken from the nursery and budded from row to row in succession year after year. Occasionally, however, bud sticks are collected in orchards. In any case, propagation of cherries takes place in late summer after the most conspicuous yellow-leaf drop stage has passed. Owing to these circumstances it is quite apparent how the yellows disease can get into nursery trees through the unwitting use of diseased scions. Besides there is the possibility of entry through the root stock.

The New York control program for cherry yellows has thus far been confined to the elimination of the disease from the nurseries (3). With this end in view a two-fold program was undertaken in 1941 involving: (1) the eradication of diseased plants already present in nursery plantings, requiring the proper training and supervision of the horticultural inspectors and (2) the selection of disease-free propagation materials, requiring the indexing and certifying of both scion variety and understock. Consequently, the development of a quick and reliable indexing technique for demonstrating the presence of the yellows virus in cherry was considered of paramount importance in the research program.

PRELIMINARY EXPERIMENTS AND OBSERVATIONS

It has been demonstrated experimentally and also observed in a nursery planting that cherry yellows propagates readily and produces symptoms in

the first year's growth from the bud, that is, with the production of symptoms in about a year's time from budding. This corresponds to the rate of transmission in orchard trees (5, 6, 7). While the details are being omitted for the sake of brevity, certain points deserve mention. On young nursery stock disease symptoms have a tendency to appear somewhat later in the season than on orchard trees, perhaps because nursery plants continue to grow later. Also on individual nursery plants symptoms may take the expression of many diseased leaves or be as inconspicuous as one chlorotic spot on a single leaf. In some cases first-year cherry grafts may first show symptoms as late as September; in others, trees presumably carrying the virus may show no symptoms the first year.

Similarly it has been demonstrated both in the greenhouse and in an experimental planting that diseased cherry buds propagate the disease in seedlings and in at least one grafted variety of peaches. On the peach the symptoms ordinarily appear the year following budding, and the expression consists primarily of a stunting in internodal growth, which crowds the leaves into clusters simulating rosettes. Following budding, areas of dead bark may appear in close proximity to the buds, and by the time symptoms are showing the following season the shoots receiving the diseased buds may be entirely dead, presumably from the yellows virus. However, a similar necrotic condition, along with rosette symptoms, has been frequently induced on peach with buds from a ring-spot disease,[1] a common contaminant in yellows-infected cherry orchards.

Besides speeding up symptom expression, it was noted that when the inoculated shoots were pruned back to the neighborhood of the diseased bud, necrosis was intensified with the dieback condition extending down the stub several inches within a month from pruning. Manifestly, much more work is needed on this phase of the study before drawing conclusions.

The discovery that the incubation period of yellow-red virosis on peach could be greatly reduced by a simple pruning technique (1) suggested testing this rapid transmission technique on cherry yellows (2).

1941 Transmission Experiments

Materials and Methods. Several test plants were used in this study. The so-called second-year Elberta peach trees were budded in the nursery and grown 1 year before being dug. The yearling peach seedlings had grown from seed one season in the nursery. After digging in late autumn they were potted and placed out doors for 2 months before forcing in the greenhouse. The yearling peach grafts were part of the same lot of seedlings that were budded to Elberta in early September.

The small peach seedlings were started from seed (southern, natural) and ready for germination after storage for 10 weeks in moist peat at 5° C. After germination in sand, the seedlings were potted in soil and reached the desired height (about 18 inches) in approximately 18 weeks from the start

[1] Besides ring spot, the sour-cherry-virus complex probably involves still other diseases about which too little is known at present for reliable identification.

of the cold treatment. Lots of several hundred pits each were started at monthly intervals so as to provide a supply of seedlings throughout the year. The seedlings were selected for uniformity and kept free from branches on the lower two-thirds of the stem prior to being used.

Two severe insect pests—red spider and broad mite—encountered in the greenhouse were kept in check by a routine spray application of NNOR 1–400 at weekly intervals during the summer months. No spray injury resulted from repeated use of this material when applied early in the day.

The virus was obtained principally from diseased Montmorency cherry trees in Western New York, although inoculum for one of the transmission experiments also came from 3 other susceptible varieties. Depending on the season, it was taken from dormant and actively growing trees in nursery and orchard as indicated in the several experiments. The so-called "diseased sources" usually consisted of bud sticks obtained from separate trees in one or more orchards or nurseries. The dormant bud sticks were soaked in water for 2 days before use.

The ordinary inoculation technique consisted in inserting cherry buds about midway up the stem of the growing peach seedling and wrapping with "sterilastic" bandage. For rapid transmissions (2) the stem was cut off 1 node above the diseased bud from 0 to 7 days after budding.

Results of Transmission Experiments

Experiment 1. The first bud-transmission test from cherry to peach resulted in the production of ring-spot symptoms within 2 weeks from budding. It employed second-year Elberta peach grafts and a variation of the rapid transmission technique, especially useful with older plants, which consisted of placing the dormant diseased cherry bud on one side of the stem between 2 rapidly growing young shoots. The plants had previously been pruned to about 12 inches above soil level when brought into the greenhouse and, after shoot growth started, all but the desired shoots were removed and the plants budded. Budwood from 3 different sources were each tested on 2 peach seedlings on March 6. Four unbudded plants were kept as checks. Ten days later faint chlorotic rings were beginning to appear on the leaves of one shoot underneath the diseased bud on one plant and by the 14th day became more definite. At the end of 20 days many of the chlorotic rings had become necrotic and symptoms were showing also on the shoot above the diseased bud (Fig. 1, D). Only 2 of the 6 original plants showed these symptoms, and, as time passed, the chlorosis had not spread to the newer formed leaves and, although there was, in comparison to the checks, a marked stunting of growth on all 6 plants, stunting alone was not considered a positive symptom.

Experiment 2. An exploratory experiment was next conducted that resulted in the transmission of cherry-yellows virus from cherry to peach with the production of rosette symptoms within a month from budding. It employed yearling peach seedlings as test plants and Montmorency cherry

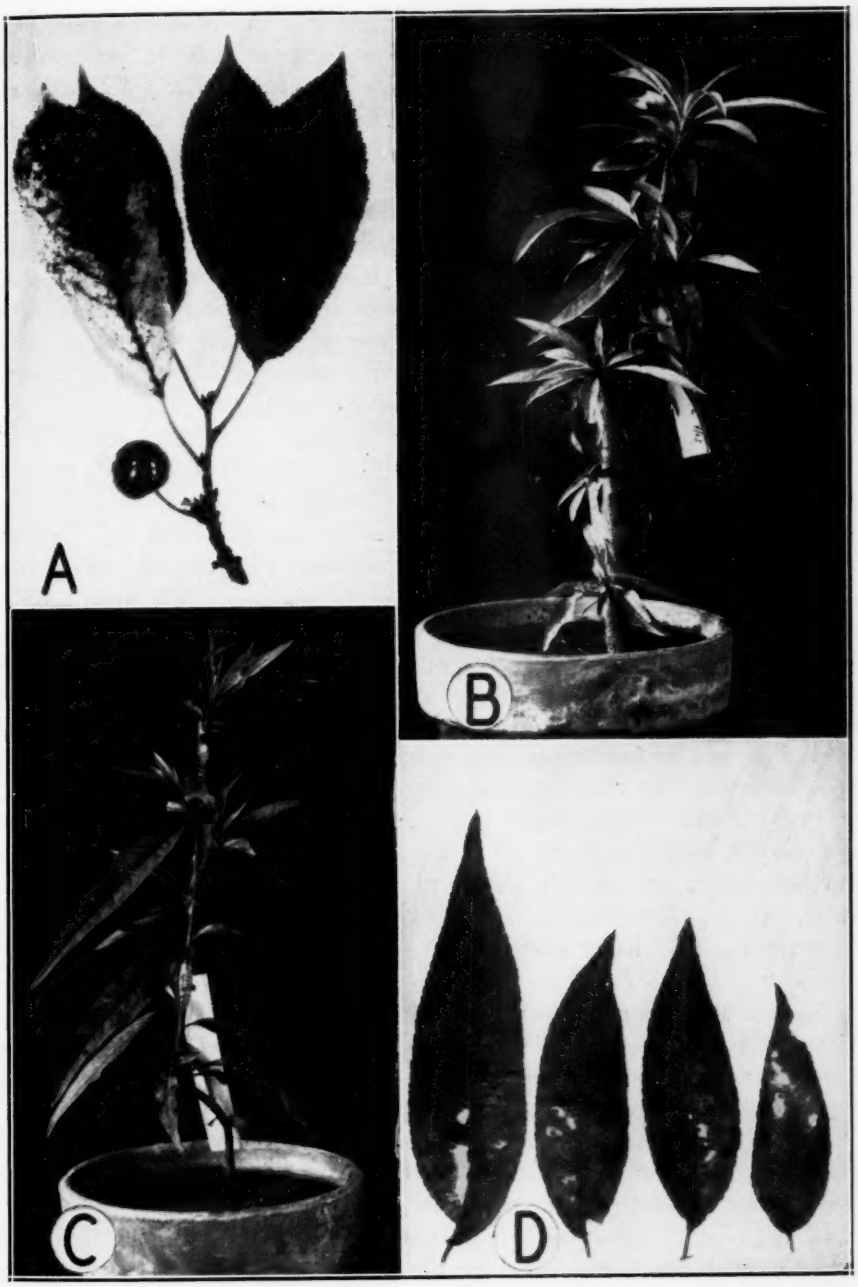

FIG. 1. A. Characteristic shoot condition late in July on a Montmorency cherry tree severely affected with yellows. Four yellow leaves were removed by the main wave of defoliation several weeks previously. B. Characteristic stunting or rosette symptoms on seedling peach 11 weeks after budding from Montmorency cherry affected with yellows. This condition persisted throughout the season. C. Typical early stage symptoms of chlorotic ring spot and rosette induced on seedling peach three weeks after budding from cherry. This plant was pruned on the day of budding. D. Chlorotic followed by necrotic ringspot symptoms induced on Elberta peach by bud inoculation from Montmorency cherry affected with yellows.

budwood from 1 healthy and 16 diseased sources. Of the diseased samples, 11 were from dormant trees collected in New York orchards, 4 from growing 2-year-old cherry trees previously inoculated by grafting from 1 New York and 3 out-of-State sources, and 1 from a cherry yellows infected peach seedling in the previous experiment.

On April 23, 3 seedlings were budded from each of 16 diseased trees from 8 different orchard sources, and 8 from a healthy nursery source. The point of bud insertion was about 12 inches above soil level on either the main stem or a side branch. Seven days later 2 of the 3 trees receiving the diseased buds and all 8 receiving healthy buds were cut back to 1 node above the inserted bud to induce new growth. For purposes of comparison, 9 yearling Elberta grafts received diseased buds from 1 source and at pruning time 3 were left unpruned.

Definite symptoms were first observed on 10 of the inoculated plants 18 days after pruning or 25 days from budding. The new spurt of growth stimulated by pruning showed occasional leaves with chlorotic rings, some of which became necrotic and later fell out, leaving perforations. At this stage the internodal growth was already definitely shorter and the leaves smaller in the inoculated plants than in the checks. Subsequently, the chlorosis disappeared while the stunting of growth persisted. A rather marked rosette condition, which persisted throughout the season, is shown in figure 1, B. This plant was photographed on July 15 or slightly under 3 months from budding. The stunted rosette condition was not always so marked as in this illustration nor the leaves so chlorotic. In some cases chlorosis was totally absent and rosetting still very pronounced.

Positive transmission, based primarily on the stunted rosette symptom, was obtained in 1 or more plants in 15 out of the 16 diseased sources tested. Three months from the start of the experiment symptoms were evident in no case in the unpruned inoculated trees or in the checks receiving buds from the healthy cherry sources. The uninoculated check trees all grew normally.

Similar but less striking results were obtained with the Elberta peach grafts. However, only 2 of the 6 inoculated trees showed positive symptoms. Since more of these plants were not available, the phase of the work with grafted varieties was discontinued.

Of interest was the observation that both of the peach seedlings receiving diseased peach buds became diseased. This indicates that cherry-yellows virus can be transmitted from peach to peach.

Experiment 3. The third bud-transmission experiment, which employed young 18-inch peach seedlings and the pruning technique, resulted in the production of positive symptoms in 23 days from budding. This experiment, which involved the indexing on peach seedlings of diseased cherry material from 4 sour-cherry varieties, gave more striking results than were obtained on the older seedlings in previous experiments. The cherry material used in this experiment was collected from 2 nurseries and 1 orchard

in July, and consisted of 7 diseased, 2 doubtful, and 1 healthy sources. Of the diseased-bud sources 4 were Montmorency, and 1 each of Early Richmond, Chase, and English Morello. The peach seedlings were started from seed and budded when they were large enough to receive cherry buds about midway on the stem or when about 18 inches tall. Two trees received buds from each source. Four check trees received buds from the healthy source. Bud inoculations were made on July 25, and the tops were pruned back to 1 node above the diseased bud on the following day.

FIG. 2. Two diseased and one check peach seedling showing the stunting or rosette symptom typical of what obtained four to six weeks after inoculation when the rapid transmission technique was used for indexing cherry yellows buds on peach.

Disease symptoms were beginning to appear on the 21st day, and all the trees receiving buds from diseased cherry trees developed the typical symptoms of chlorotic followed by necrotic rings and rosette. Negative results were obtained in the 2 cases with doubtful material and in the 4 checks. A typical rosette condition at the end of 4 weeks is shown in figure 2. Note the difference in length of growth between the diseased trees and the check.

Experiment 4. In experiment 4 the budwood was collected from 6 different cherry orchards in August and tested on small peach seedlings, as in experiment 3, with similar results (100 per cent infection). Three trees were budded from each of 6 diseased and 1 healthy sources. Pruned uninoculated check trees also were included. The plants were budded on August 19, pruned back to 1 node above the diseased bud 5 days later, and were showing the characteristic early symptoms of chlorotic ring spot and rosette within 3 weeks. The plants receiving the buds from the healthy source grew normally. All yellows buds used in this experiment came from known severely diseased trees. While removing the leaves long pedical stubs were left to mark the location of the yellow leaves on the bud sticks, and buds from these locations were used exclusively.

Experiment 5. The fifth indexing experiment tested 3 sources of diseased buds from orchards each budded on 5 different seedlings with results practically identical to those obtained in experiment 4. The 5 trees receiving healthy buds were negative.

Experiment 6. The final indexing experiment involved budding 2 trees each from 2 sources—1 diseased and 1 healthy—and pruning immediately afterward. The buds were inserted on September 18 and the first symptoms, consisting of chlorotic rings and rosette, were evident 17 days later. Pruning immediately after budding seemed definitely to have shortened the incubation period in this experiment. One of these diseased seedlings, photographed at the end of 3 weeks, showed typical early stage symptoms (Fig. 1, C).

Thus, by means of the rapid transmission technique, it has been found possible to collect cherry budwood from trees showing yellows symptoms and to index and, therefore, certify as to the presence or absence of cherry-yellows virus within a month's time.

Because of its rapidity this technique has made it possible to select sources of propagation material in early summer and then to index same so as to obtain results before the regular budding season late the same summer.

After the initial chlorosis, which usually disappears, the severe stunting or rosette condition ordinarily persists throughout the remainder of the season (Fig. 1, B). However, there were exceptions in which the initial symptom stages were followed by a partial recovery suggesting some variability in the virus itself. Infrequently some stunting occurred in occasional peach seedlings receiving buds from apparently healthy cherry trees. To further complicate the picture, stunting has also been induced in peach seedlings by several other cherry viruses now under investigation. It is obvious, therefore, that much additional work will be needed to clarify the broader problem of cherry viruses.

Apparently the rapid transmission technique has definitely demonstrated its reliability for identifying severely diseased trees, but it will remain for future studies to determine its accuracy and reliability with cherry trees in the early stages of disease.

This technique, because it is not exacting for materials, space, or time, should prove generally useful for greenhouse studies, and probably for outdoor work, with proper care and attention to details. It also has possibilities in studies on other viroses (2).

SUMMARY

The cherry-yellows virosis has been transmitted by budding and grafting from diseased to healthy cherries with the production of definite yellow-leaf symptoms within approximately 1 year.

It also has been transmitted by budding in late summer from diseased cherries to healthy peach seedling trees with the production of dieback and a stunting rosette condition the following growing season. When the shoot above the bud was removed from 4-year-old Elberta peach trees, about a week after budding, instead of inducing new shoot growth the stub died back several inches below the diseased bud within a month from the pruning operation.

In the first indexing experiment, which involved placing diseased cherry buds between two rapidly growing shoots on cutback second-year Elberta peach grafts, chlorotic ring-spot symptoms were induced within 2 weeks from budding.

Buds from cherry trees affected with yellows indexed on yearling peach seedlings and cut back to 1 node above the diseased bud, resulted in the production of symptoms consisting of ring spot and rosette within a month from budding.

When repeated on small 18-inch-tall seedlings, shorter incubation periods of about 3 weeks were obtained in 4 different experiments.

This rapid indexing technique offers promise in advancing the work on cherry yellows and, in some modification, also may prove generally useful in studies on other stone-fruit viruses.

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AN UNUSUALLY VIRULENT RACE OF WHEAT STEM RUST, NO. 189

G. GARCIA-RADA, J. VALLEGA, W. Q. LOEGERING, AND E. C. STAKMAN[1]

(Accepted for publication December 18, 1941)

A hitherto undescribed race of *Puccinia graminis tritici*, extraordinarily interesting and important in several respects, has been identified from rusted Khapli emmer collected in Peru. This race, numbered 189 in the key of Stakman and Levine (7, 8), can cause heavy infection on all the differential wheat varieties used in identifying races. No other known race has this ability, the nearest approach being race 15, which attacks all but Khapli emmer. Four other races, Nos. 41, 42, 72, and 99, can cause moderate to rather heavy infection on seedlings of Khapli, but only under very favorable environmental conditions. Race 72 causes a type-X infection, indicating a considerable range in size of uredia; race 99 produces type 3, which indicates moderate susceptibility; and races 41 and 42 produce type 3 to 4, usually with pronounced chlorosis or even a tendency to necrosis around the uredia. None of these 4 races have been found in nature in the United States or Mexico. Race 41 was isolated from Egypt; race 72 has been obtained only from artificially made crosses in the greenhouse; and race 99 has been isolated only from South Africa, by Verwoerd (12). Race 42 was first isolated by Stakman and Levine from rusted wheat grown in Egypt and was subsequently found by Vallega (10) to be rather common in Argentina and neighboring regions. In studying factors affecting the development of this race on Khapli, Vallega found that it caused a wide range in infection type, both on seedlings and adult plants, depending on environmental conditions; it causes really heavy infection only under optimum temperature and light conditions. Race 189, on the other hand, does not require especially favorable conditions but can attack seedlings and adult plants of Khapli heavily under a rather wide range of conditions. It also can attack seedlings and adult plants of *Triticum timopheevi*, hitherto resistant to virtually all races, and is quite virulent on Hope wheat, one of the most generally resistant of all adequately tested varieties.

That race 189 is of practical importance is shown by the fact that Khapli emmer, grown commercially for a number of years in coastal regions of Peru because of its resistance to stem rust when other varieties tested were susceptible, has been very severely injured by rust for at least two successive

[1] The experiments on which the results reported in this paper are based were made cooperatively by G. Garcia-Rada, of the Estacion Experimental Agricola de La Molina, Lima, Peru; J. Vallega, of the Instituto Fitotecnico, Llavallol, F. C. S., Argentina; and members of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, cooperating with the Minnesota Agricultural Experiment Station.

The writers are indebted to Dr. M. N. Levine and Dr. E. A. Ausemus for help in obtaining some of the data for the paper.

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years. Several years ago Abbott (1, 2, 3) stated that Khapli emmer and Hope wheat might rust heavily in Peru. He concluded that a new and unusually virulent race of *Puccinia graminis tritici* was present, as all the differential varieties of Stakman and Levine were susceptible when inoculated in the greenhouse with rust from Khapli emmer. Several attempts were made later to identify this Peruvian rust at the Federal rust laboratory, St. Paul, Minn., but the spores were nonviable in all the collections obtained. In 1939 one of the writers (Garcia-Rada) called attention to the fact that Khapli had been severely injured by rust in certain wheat-growing localities in the coastal region of Peru (Fig. 1). In the same year he sent uredial

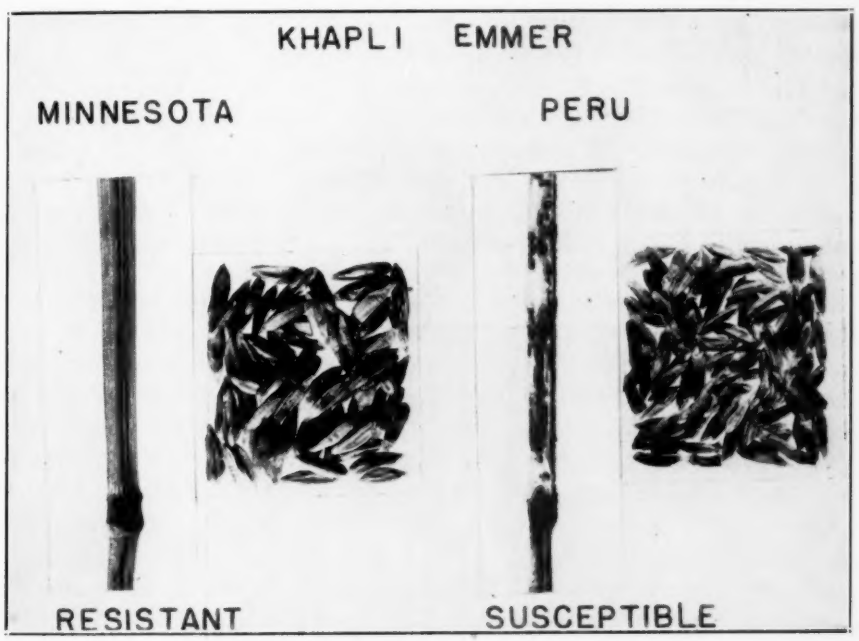

FIG. 1. Khapli emmer, resistant to rust in Minnesota (left) and damaged by rust in Peru (right).

material to Vallega at the Instituto Fitotecnico at Llavallol, F. C. S., Argentina, and to the Federal rust laboratory at St. Paul, Minn. Infection was obtained with the material sent to Argentina, but spores of the sample sent to St. Paul were not viable on arrival. The infection types produced on the differential varieties by the rust sent to Argentina were as follows:[2]

[2] In the system of Stakman and Levine (7), infection types range from 0 to 4, 0 indicating immunity, 1 and 2 indicating resistance, and 3 and 4 indicating susceptibility. The uredia in type 4 are larger than those in type 3 and have a greater tendency to coalesce. Sometimes there are imperceptible gradations between types, especially types 3 and 4, and the deviations from the standard of the type are shown by plus or minus signs. The letter c simply shows that there were pronounced chlorotic areas around uredia. There is a sixth type, designated X, in which individual uredia represent a considerable range of types.

Hood[a]	4	Spelmar	4
Marquis	4	Kubanka	4
Reliance	4	Acme	4
Kota	3+	Einkorn	4
Arnautka	4	Vernal	4
Mindum	4	Khapli	4[c]

[a] Substitute for Little Club.

It was evident from these infection types that the collection comprised a new physiologic race (11). In September, 1940, one of the writers (Stakman) visited the experiment station at Lambayeque, Peru, at the suggestion of Garcia-Rada, and was shown heavily rusted plots of Khapli emmer, Hope wheat, *Triticum timopheevi,* and several other varieties that had been highly resistant for a number of years in many widely separated geographic regions. Some of the rusted Khapli was sent by airmail to St. Paul, Minn., through the Division of Foreign Plant Quarantines, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and inoculations were made later when there was no danger of spores being disseminated to growing vegetation. A pure culture was obtained of a race that attacked all the differential varieties heavily, thus agreeing with Vallega's results and confirming Abbott's earlier conclusion regarding the existence in Peru of an unusually virulent race, to which the number 189 has now been assigned (Fig. 2).

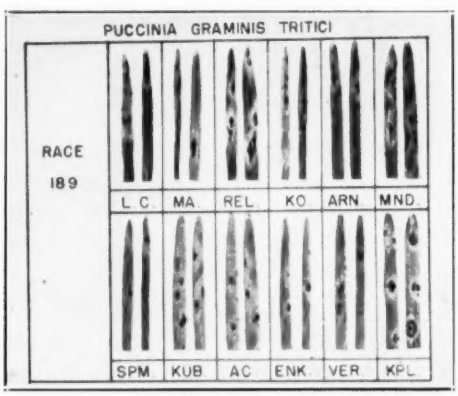

FIG. 2. The effect of race 189 on the differential host plants.

Although the work with this collection was done during the dormant season, so that there was no opportunity for spores to be blown to growing vegetation, every possible precaution was taken between the arrival of the collection and the time when it was completely discarded. All inoculated plants and the soil and pots in which they were grown were disinfected in a large steam sterilizer under 15 pounds of pressure for 2 hours. Inoculations were made in an isolation booth, and the greenhouse benches, partitions, and walls of the isolation booth were dusted with sulphur and later washed with copper sulphate solution.

Race 189 is very virulent not only on seedlings in the greenhouse but also

on adult plants in the field. Seedlings of about 50 resistant varieties and selections recently produced in the United States were at least moderately susceptible when inoculated in the greenhouse. More important, however, is the ability of race 189 to cause heavy damage, in the field, to adult plants of varieties such as Khapli, Hope, and *Triticum timopheevi* that have adult-plant resistance in addition to their protoplasmic resistance to many physiologic races. As far as the writers are aware, Khapli, for example, has not been rusted heavily anywhere except in Peru, where in some years and localities it would have been considered a completely susceptible variety by any one unfamiliar with its record elsewhere. It is true that weather conditions for rust development are likely to be favorable in the coastal region of Peru because of the abundance of fog; but the most important factor in the susceptibility of Khapli and other hitherto resistant varieties seems to be the presence of race 189.

Stakman and Hart (6) emphasized the fact that the resistance of wheat varieties to stem rust, especially in the field, is determined by a complex series of interacting factors, genetic and environmental, involving the host, the particular race of the pathogen, and the interaction between the two. It is evident that certain otherwise resistant varieties cannot withstand the combination of a very virulent rust race and favorable rust weather in at least part of the coastal region of Peru. Possibly it is futile to attempt to assess the relative responsibility of the weather and of the pathogen. Obviously, even so virulent a race as 189 requires favorable weather for infection and subsequent development, but even the most favorable weather will not enable nonvirulent races to attack resistant varieties. Khapli emmer, for example, has remained resistant in the United States from 1914 to 1941, inclusive, even in the most devastating natural and artificially induced stem rust epidemics.

Khapli was introduced into the United States from India in 1908 (4), has been tested in certain experimental plots and rust nurseries for about 30 years, and for about 20 years has been grown in the uniform rust-observation nurseries of the U. S. Department of Agriculture at an average of about 50 stations in different regions of the country. Furthermore, there are records of its performance in or adjacent to the rust-breeding nursery at University Farm, St. Paul, Minn., each year from 1914 to 1941, inclusive.[3] In this nursery, epidemics are induced artificially by inoculating with most of the races of wheat stem rust that occur in nature in the United States. And yet the rust readings on Khapli, even under such exceptionally severe conditions, have been "trace" every year except 1916, 1922, 1925, and 1926. In 1916 the reading was 50± per cent, while that for the susceptible Little Club was 95 per cent; in 1922 the reading was 10R, and in 1925 and 1926 it was 5R. The figures represent the percentage of what is considered the

[3] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Agronomy and Plant Genetics and Division of Plant Pathology and Botany, Minnesota Agricultural Experiment Station.

maximum possible number of pustules, and "R" indicates "resistant." In only one year of the 28, then, was there a really appreciable amount of rust on Khapli. In 1916, when one of the worst epidemics on record developed in the spring-wheat region of the United States, there were about half as many pustules on Khapli as on the most susceptible varieties, but they were small.

One of the writers (Stakman) has studied and observed Khapli for more than 30 years. It was one of the varieties studied in early attempts to learn something about the nature and variability of resistance (5) and is one of the differentials used in identifying physiologic races. During the past 20 years seedlings of Khapli have been inoculated with rust from more than 10,000 collections of wheat from the United States and Mexico, but have been highly resistant to every one of them. Khapli has therefore been inoculated with all physiologic races identified during that time but has not been susceptible, even in the seedling stage, to any race ever found in nature in the United States and Mexico. Furthermore, the same writer has seen Khapli in field plots at many widely separated stations in many different years in the United States but has never seen large pustules or evidences of injury. Environmental conditions certainly have been favorable for the development of rust in the thousands of tests in the greenhouse, because susceptible varieties rusted heavily; conditions were favorable for rust development in the rust nursery at St. Paul, Minn., where there has been an epidemic, artificially induced and fostered, each year for many years; and there have been several devastating and widespread natural epidemics and a number that were destructive locally. Obviously, then, Khapli has been very extensively tested against North American rust races, often under ideal conditions for rust development, and has been one of the most highly and consistently resistant, along with Hope and *Triticum timopheevi,* of all varieties tested.

The complete susceptibility of Khapli in Peru, therefore, appears to be due primarily to the prevalence of the especially virulent race 189, and the same appears to be true of Hope wheat and *Triticum timopheevi.* This supports the conclusion that, although some varieties of wheat have a combination of factors for resistance that enables them to withstand destructive attacks of stem rust in most seasons and in most regions, nearly all known varieties may sometimes become more or less heavily rusted by some rust races, in some regions and under certain combinations of environmental conditions. Consequently the importance of continuing the attempt to find and combine the most generally effective genes for resistance to stem rust seems obvious.[4]

Race 189 has been found only in Peru, where it appears to be distributed in the coastal region from Lambayeque on the north to Lima on the south.

[4] It appears now that a few hybrid selections are at least partly resistant in Peru, even in the presence of race 189, the most generally virulent race of wheat stem rust now known; but further tests are needed, and, in any case, a detailed account of varietal behavior is beyond the scope of the present paper.

Whether it is more widespread in Peru is not known. It has not yet been found elsewhere in South America and has never been isolated either from wheat or barberry bushes in the extensive physiologic-race surveys that have been made annually in the United States for about 20 years. It seems unlikely that it would have been missed had it been present even in small amounts. Supporting evidence that it does not occur in North America is the fact that Khapli emmer has been resistant for about 20 years in approximately 50 uniform rust nurseries in widely separated places in the United States.

Whether urediospores of this very virulent and dangerous race can be carried by the wind from Peru to other countries of South America or to North America is an important question. If it is now restricted to the coastal region of Peru, the Andes mountains constitute a formidable but not necessarily impassable barrier to its eastward spread. As the present distribution seems to be south of the equator, the likelihood that inoculum might be blown to North America may seem somewhat remote, although it is possible that the rust might spread northward across the equator by easy stages on grains or grasses at high elevations and become established in northwestern South America, from which spores might possibly be carried by the wind to Central America, Mexico, and grain-growing regions of the United States and Canada. In any event it seems important to ascertain the extent to which urediospores are disseminated by the wind in equatorial regions and particularly whether they can be carried from one continent to another.

Nothing is known regarding the time and mode of origin of race 189. There is circumstantial evidence that it has been present in Peru for at least 15 or 20 years, possibly longer. New races of *Puccinia graminis* are known to result from recombinations in the sexual stage on *Berberis* spp., the alternate hosts of the rust. Native barberries are numerous in certain parts of the Sierra of Peru and they are now known to become infected. Although they may not be important in initiating annual outbreaks of rust, it seems certain from experience elsewhere (9) that they probably are important in the perpetuation of physiologic races and the production of new ones. Race 189 never has been isolated from the aecial stage on barberries in North America nor has it been obtained from artificial crosses. Possibly the combination of genes represented in this race is rarely made. On the other hand, it has been made and may be made again. At all events, the existence of so virulent a race is definite confirmation of the statement so often made in the past that nature can produce very virulent races of rust just as man can produce very valuable varieties of wheat. Therefore, it is important to study the prevalence and distribution of physiologic races, eradicate the alternate host where feasible, and continue breeding work to find and combine genes for resistance in the most effective manner possible.

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A STEM-END ROT OF POTATO TUBERS CAUSED BY RHIZOCTONIA SOLANI[1]

F. S. THATCHER[2]

(Accepted for publication December 5, 1941)

Rhizoctonia solani is widely considered to be a species comprised of many physiological races, and is recognized as a parasite of an unusually extensive host range. Nevertheless, reports of the decay of normal potato tubers by this organism are uncommon. Shapovalov[3] has described a "stem-end decay of the jelly type" caused by *Rhizoctonia solani,* but in this instance each affected tuber was one with an abnormally elongated stem-end, the decay being confined to this region, which was characterized by "a striking deficiency in starch." Since preparation of this paper, Storey[4] mentions having isolated *R. solani* from a "tuber showing jelly-end-rot," but was unable to duplicate symptoms by artificial inoculation.

Potato tuber specimens showing a stem-end decay of a "punky" consistency were submitted recently to the writer from a locality in Prince Edward Island. *Rhizoctonia solani* was isolated from these specimens and its pathogenicity proved by inoculation tests.

The disease apparently originated at the stolon scar, involving parenchyma tissue radially from that point for a distance of 1 to 3 cm., and was finally evident as a dark necrosis with an abrupt margin. The external appearance of the specimens was thus strongly suggestive of the stem-end rot caused by *Fusarium solani* var. *eumartii* (Fig. 1, A–C).

When first examined, the necrotic tissue was of a resilient, "punky" or "cheesy" consistency. On exposure to laboratory air the lesions soon became sunken and, whenever the periderm was broken, the diseased tissue dried out quickly and shrivelled.

The microscope reveals the typical brown hyphae of the pathogen dispersed among the cells of the diseased parenchyma tissue. Ultimately, the progress of the fungus is restricted by extensive phellogen formation in the healthy tissue just beyond the margin of the necrotic zone (Fig. 2, A and B). The phellogen, and the suberized cells developing from it, are not always effective as a barrier, in which case the lethal action of the fungus continues until another phellogen layer surrounds the secondary necrotic region (Fig. 2, B). In the specimens examined the cell walls of the more recently invaded tissues were intact, but necrotic cells a few cell diameters away from the border of the affected region had undergone cell wall disintegration to such an extent that the residuum was little more than a mass of

[1] Contribution No. 687 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Junior Plant Pathologist.

[3] Shapovalov, M. *Rhizoctonia solani* as a potato-tuber rot fungus. Phytopath. 12: 334–336. 1922.

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starch grains, of which there was the normal complement. There was no indication that the fungus induced hydrolysis of starch grains, as is evident in figure 2, D. Figure 2, A and B, illustrate clearly the disintegration of cell walls, which is the change that probably accounts for the "punky" nature of the necrotic mass.

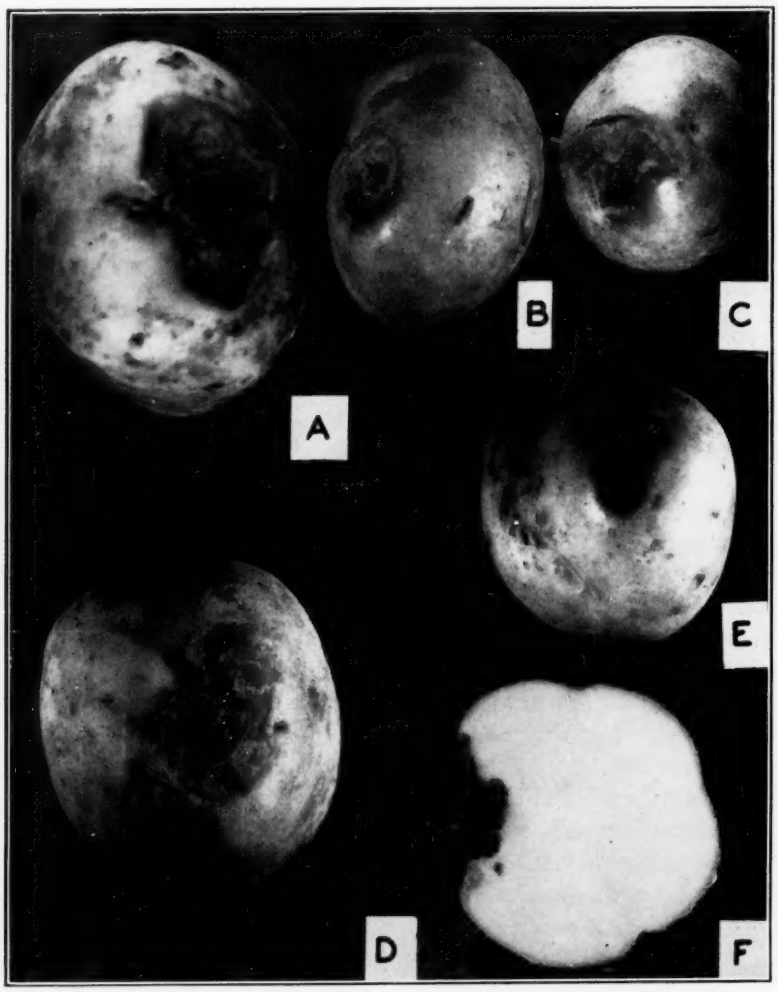

FIG. 1. A–C. Stem-end decay caused in the field by *Rhizoctonia solani*. × ⅔. D–F. Stem-end decay caused by *R. solani* following artificial inoculation; tuber kept in wet sand at 15–20° C. after inoculation. E. Tuber held at 5° C. after inoculation.

Sections from tissue embedded in paraffin reveal that the tracheids are the probable path of entrance of the pathogen. Hyphae were found to be plentiful within the lumina of tracheids present in the vascular bundles leading from the stolon scar, the hyphae sometimes being detected in the tracheids in tissue beyond any visibly necrotic parenchyma. At intervals along the path of the bundle the hyphae escape from the tracheids *via* pits

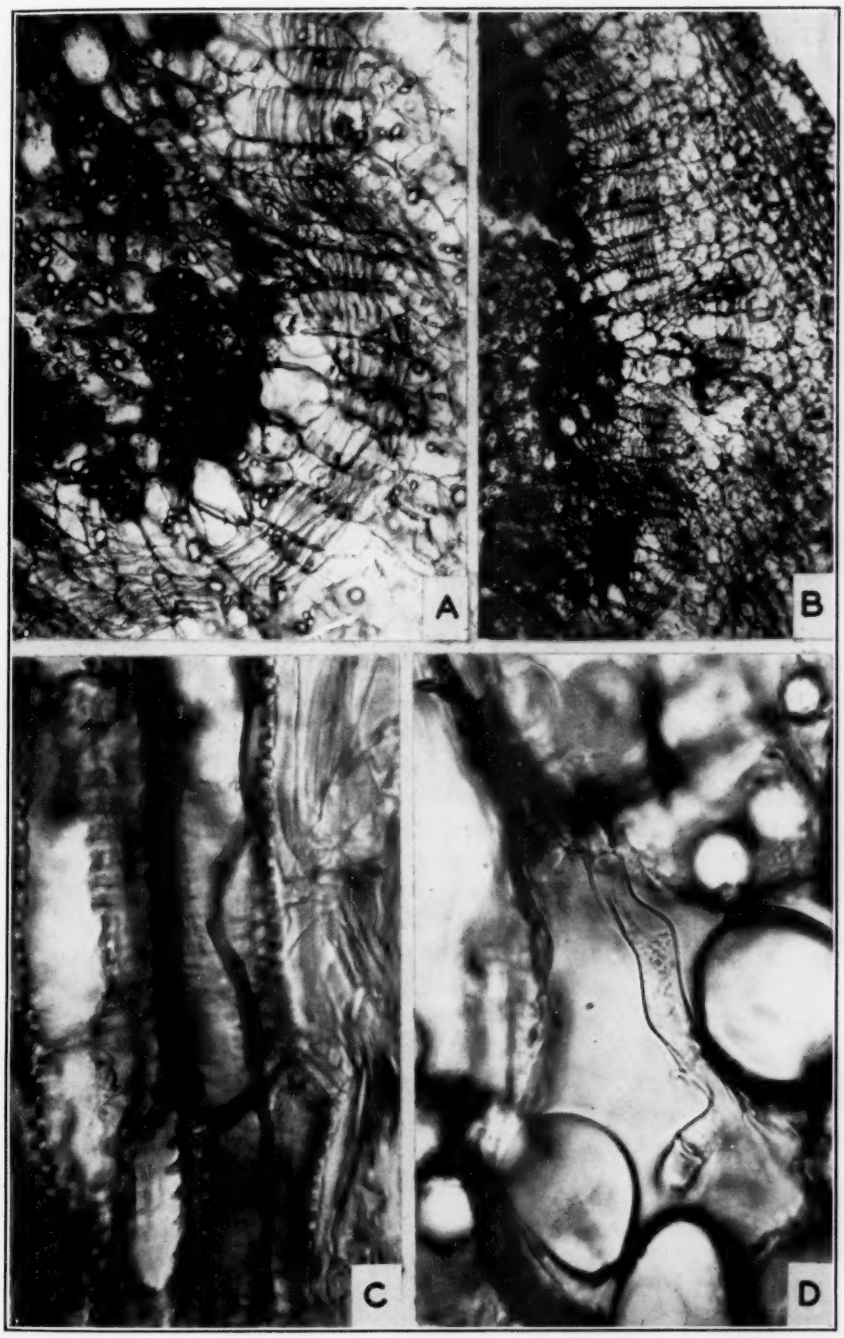

FIG. 2. Sections of potato tuber tissue infected by *Rhizoctonia solani*. A. Extensive phellogen development surrounding necrotic tissue. ×100. B. Necrotic tissue surrounded by a phellogen, which proved to be an ineffective barrier to the fungus. The small region of secondary decay is being surrounded by another phellogen layer. ×40. C. Hyphae of *R. solani* present in the lumina of tracheids. ×1,000. D. Starch grains in decaying tissue which show no sign of being hydrolyzed by the fungus. ×1,500. A, B, and D. Freehand, unstained sections. C. Paraffin section, stained by safranin and fast green.

into parenchyma cells, whence typical cell degeneration occurs. This explains the fact that the furthermost regions of disintegrating parenchyma are evident as short cones of necrotic tissue centered about groups of infected tracheids. *Rhizoctonia* mycelium within the lumen of a tracheid is seen in figure 2, C.

Very wet and cool weather prevailed just before harvest in the locality in which the diseased specimens had been found. Under these conditions of low temperature and low oxygen-tension (the latter brought about by the soil being saturated with water), a wound periderm is formed very slowly at the stolon scar and the ends of the vascular bundles leading from the stolon are not effectively occluded. Hence, the *Rhizoctonia* hyphae are able to grow from the decaying stolon into the tuber tissues and are able to continue growth until such time as conditions favor wound-periderm formation. The appearance of the specimens seemed to indicate that this was not complete at time of digging.

The results from pathogenicity tests support the suggestion that *R. solani* can cause decay in the potato tuber unless prevented by rapid wound-periderm formation. Green Mountain tubers that had been in storage for a month were inoculated by inserting mycelium from a 2-week-old culture growing on potato-dextrose agar into a small incision made at the stolon scar. Some of the tubers were kept in moist chambers held at temperatures of 5, 14, and 20 degrees C.; others were covered in very wet sterile sand, kept at from 15° to 20° C. Typical symptoms developed in the tubers held at 5° C. and in those kept in the wet sand (Fig. 1, D–F). Under each of these conditions periderm formation would be slow. Tubers in the moist chambers held at 14° and 20° C. developed only a slight degree of "Stem-end browning."

FURTHER STUDIES ON THE TEMPERATURE RELATIONS OF SCLEROTIAL ISOLATES OF RHIZOCTONIA SOLANI FROM POTATOES[1]

E. L. LeClerg, L. H. Person, and S. B. Meadows[2]

(Accepted for publication March 13, 1942)

INTRODUCTION

Previous investigations[3] on the relation of temperature to radial growth on artificial media of 20 isolates of *Rhizoctonia solani* Kühn from sclerotia on potato tubers indicated an optimum at 25° C. or lower. Under identical conditions, the optimum temperature for 19 of 20 crown-rot isolates from sugar beets was 30° C., one isolate growing slightly faster at 25° C. In further work,[4] it was found that the optimum temperature for radial growth of dry-rot isolates from sugar beets was also 30° C., whereas it was 25° C. for 4 additional sclerotial isolates from potato tubers.

One of the writers (Person) had occasion to isolate this fungus from sclerotia on a large number of potato samples from several States producing certified-seed. These isolates formed the basis for this study, which was made to supplement the investigations mentioned above.

EXPERIMENTAL RESULTS

The influence of temperature on radial growth of 63 sclerotial isolates from potatoes, two crown-rot isolates from sugar beets, and 2 dry-rot canker isolates from sugar beets was studied at 20, 25, and 30° C. The data presented are averages of duplicate tests each of which consisted of 2 cultures on potato-dextrose agar at each temperature. Agar discs, of a uniform size cut out with a sterilized cork borer, were used for inoculum.

From the data in table 1, it appears that 25° C. was the optimum temperature for all of the potato sclerotial isolates. The optimum temperature for the sugar-beet crown-rot and dry-rot isolates, however, was 30° C. These results confirm those previously reported.[5]

In making isolations of sclerotia from tubers more than one sclerotium from a tuber was planted on agar. In table 2 is indicated the radial growth on potato-dextrose agar of two isolates from each of four potato tubers at 20°, 25°, and 30° C. It is apparent that sclerotial isolates from the same tuber may differ in rate of radial growth at the several temperatures.

[1] The data herein presented were obtained in cooperative investigations by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Departments of Botany, Plant Pathology, and Bacteriology, and of Horticultural Research of the Louisiana Agricultural Experiment Station.

[2] Pathologist of the Division of Fruit and Vegetable Crops and Diseases, United States Department of Agriculture, Assistant Pathologist of the Louisiana Agricultural Experiment Station, and Agent of the Division of Fruit and Vegetable Crops and Diseases, United States Department of Agriculture, respectively.

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[5] See footnotes 3 and 4.

TABLE 1.—*Optimum-growth temperature for isolates of Rhizoctonia solani from sclerotia on potatoes and from crown-rot and dry-rot lesions of sugar beets, as indicated by the number of isolates with maximum radial growth at the indicated temperature*

Source	Number of isolates tested	Number of isolates with optimum temperature at		
		20° C.	25° C.	30° C.
Sclerotial isolates from potatoes				
Nebraska	38	0	38	0
North Dakota	18	0	18	0
Maine	4	0	4	0
Colorado	2	0	2	0
Michigan	1	0	1	0
Crown-rot isolates from sugar beets				
Michigan	1	0	0	1
Ohio	1	0	0	1
Dry-rot isolates from sugar beets[a]				
California	1	0	0	1
Ohio	1	0	0	1

[a] The writers are indebted to Dr. J. E. Kotila for the dry-rot isolates from sugar beets.

TABLE 2.—*Comparative radial growth at 3 different temperatures of two isolates of Rhizoctonia solani obtained from different sclerotia on each of four potato tubers*

Source of tuber	Tuber number	Average colony diameter (mm.)		
		20° C.	25° C.	30° C.
Nebraska	2a	45.0	56.0	50.2
	2b	36.2	46.5	23.5
Nebraska	32a	46.0	58.2	46.2
	32b	60.5	62.5	44.0
North Dakota	5a	55.7	62.2	46.5
	5b	55.0	57.5	43.0
North Dakota	12a	56.2	61.2	44.7
	12b	47.5	54.0	51.7

SUMMARY

Comparative studies were made to determine the optimum temperature for radial growth on potato-dextrose agar of 63 isolates of *Rhizoctonia solani* from sclerotia on potato tubers. Two crown-rot and 2 dry-rot canker isolates from sugar beets also were used. Determinations were made at 20°, 25°, and 30° C.

It was found that potato sclerotial isolates made the greatest radial growth at 25° C. The isolates from the two types of sugar beet lesions grew best at 30° C. These results confirm those previously reported by the senior writer.

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PRELIMINARY INVESTIGATIONS ON MECHANICAL INJURY IN FLAX SEED[1]

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An examination of a large number of flax-seed samples in 1940 revealed that practically all of those originating in Manitoba, Saskatchewan, and Alberta were so damaged through a cracking of the seed that, on the average, about half the seeds failed to germinate. Seed samples from Ontario, Quebec, and the Maritime Provinces usually were not injured at all. In 1941, there was less cracking of the seed, but the amount of damage was again greater in Western than in Eastern Canada. This injury to flax appeared to be similar to that described, respectively, by Stevens[2] and by Härtel[3] for the United States and Germany.

The cracking of the seed was observed to occur during threshing, the available evidence indicating that the damage was likely to be most severe when the crop was threshed in very dry weather. Ordinarily, the cracks were invisible to the unaided eye, but sometimes their presence was indicated by broken or chipped seeds. Many samples, grading high in commerce, were found to be badly damaged. Large-seeded varieties seemed to be more subject to injury than were small-seeded varieties.

When samples containing cracked seeds were planted in the greenhouse in beds of nonsterile soil, such seeds either failed to germinate or produced stunted, distorted plants, thus causing a reduction in stand, which seemed attributable to the rotting of cracked seed by soil-inhabiting micro-organisms. The rotting did not occur when the seed was germinated on moist paper, as in official tests, or in autoclaved soil. Soil temperature within the range of 10–30° C. appeared to have no effect on the amount of seed rotting in nonsterile soil, but the rotting was less when the seed was sown in light soils than in heavy soils.

Disinfection of the seed with Ceresan or Half-ounce Leytosan completely prevented the rotting of cracked flax seed in nonsterile soil. The most effective rates of application for these dusts were found to be 1½ ounces per bushel for Ceresan and 2 ounces per bushel for Half-ounce Leytosan. The average germination, for 362 flax samples collected in 1940 and 1941, was about doubled by this treatment. In seedlings from cracked but treated seed, the only evidence of former injury to the seed was found on the cotyledons, which were scarred where the seed had been cracked.

Field experiments, in 1941, with 8 varieties of flax obtained from each of

[1] Contribution No. 697 Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Stevens, O. A. Germination studies on aged and injured seeds. Jour. Agr. Res. [U. S.] **51**: 1093–1106. 1935.

[3] Härtel, K. Über druschverletzten Lein und keimverletztes Getreide und ihre Beurteilung bei der Reinheitsbestimmung. Compt. Rend. Assoc. Internat. d'Essais de Semences **2**: 213–223. 1936.

6 different stations showed that the cracking of the seed influenced the density of stand in the same way as it did in the greenhouse. The reduction in stand was proportional to the amount of seed cracking. The planting of cracked seed also reduced the yield of grain, but the amount of this reduction seemed to be affected by the location of the experiment. At Winnipeg, for instance, yields were not reduced when less than 20 per cent of the seeds were cracked. At Morden, Manitoba, where growing conditions for flax were less favorable than at Winnipeg, this amount of cracking reduced the yield. In both cases, the disinfection of cracked seed raised the yield to the level in the crop from undamaged seed. To illustrate the effect of seed disinfection on cracked seed, the results obtained from duplicate experiments at Winnipeg and Morden are shown in table 1. In these experiments, 3 different seed lots of Redwing flax were sown in 1/100-acre plots arranged in a Latin square. Full competition from weeds was allowed.

TABLE 1.—*The effect of seed disinfection with Ceresan (1½ ounces per bushel) on seed germination and yield in three lots of flax seed showing different degrees of mechanical injury*

Seed sample	Percentage increase from seed treatment				
	Germination			Yield	
	Laboratory	Field (Winnipeg)	Field (Morden)	Winnipeg	Morden
A	41.0	17.1	58.3	0.0	20.0
B	91.0	33.1	85.8	6.0	33.5
C	174.0	38.9	92.2	12.5	45.5

The results of this preliminary investigation suggest that, in comparative tests of varieties, it is important to plant undamaged seed or, if the seed is damaged, treated seed. In comparative tests of disinfectants, either mechanically-injured or diseased seed should be used. A sample of seed of a desirable variety may be sufficiently damaged to lower its ordinary yield, while in tests with seed disinfectants, the use of different lots of seed is likely to give conflicting results.

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PHYTOPATHOLOGICAL NOTES

A Blue-staining Fungus Inhabiting the Heartwood of Certain Species of Conifers.[1]—There are few records of bluestain in the heartwood of living trees. Lagerberg, Lundberg, and Melin[2] reported stain in the heartwood of a single living spruce tree (*Picea excelsa*). They isolated the causal organism and on the basis of cultural characteristics and the formation of small hyaline spores described it as a new species of *Hormodendrum, H. microsporum* (Lagerberg and Melin). Kaufert,[3] in 1935, reported bluestain in the heartwood of approximately 10 per cent of overmature balsam fir trees, the fungus apparently entering through dead branch stubs. Crowell[4] later described a dark bluestain occupying most of the heartwood of a single specimen of white spruce and one of balsam fir, and he stated that when pieces of wood were kept in a moist chamber for 5 weeks, mycelium grew out of the stained wood but formed no spores. His excellent illustration leave little doubt that he was dealing with the same fungus previously mentioned by Kaufert and discussed in the present note. Scheffer and Lindgren[5] state in a footnote that, ". . . also certain uncommon bluestains have been encountered developing only in the heartwood of living trees."

During the last few years the writers have cut and dissected more than 50 northern white cedar trees (*Thuja occidentalis*) in the forests of Northern Minnesota, and have found bluestain in the heartwood of every one. It usually is in streaks above and below branches or branch stubs, and sometimes occupies a considerable volume of the heartwood. Numerous isolations from the stained wood have in almost every case yielded a non-sporulating, very slow growing fungus with dark hyphae, which is the organism chiefly or solely responsible for the stain in the wood, plus one or more sporulating fungi, as yet unidentified. Typically, when isolations are made on malt- or potato-dextrose agar, one sees macroscopically only the dark, slow-growing fungus. Macroscopically, the culture looks pure. In more than a hundred isolations made on different agar media the bluestaining fungus never has been obtained alone, but always in association with other organisms. Pure cultures of the stain fungus were obtained by cutting hyphal tips on hanging agar drops. Approximately 20 cultures made in this way were very similar to each other and only slightly different from the original cultures containing the two or more organisms.

Pieces of white cedar heartwood were sterilized in an autoclave and in-

[1] Paper No. 1992 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Lagerberg, T., G. Lundberg and E. Melin. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvardsfor. Tidskr. **25**: 145–272, 561–739. 1927–28.

[3] Kaufert, F. H. Heartrot of balsam fir in the Lake States with special reference to forest management. Minn. Agr. Exp. Stat. Tech. Bull. 110. 1935.

[4] Crowell, Ivan H. Heart bluestain of white spruce and balsam fir. Pulp and Paper Magazine of Canada **41**: 451–452. 1940.

[5] Scheffer, T. C., and R. M. Lindgren. Stains of sapwood and sapwood products and their control. U.S.D.A. Tech. Bull. 714. 1940.

oculated with pure cultures of the stain fungus. The fungus grew over the surface of and through this wood somewhat slower than it grew on agar, staining the wood rather dark blue. The mycelium is not restricted to particular cells or tissue systems of the wood, but grows through tracheids and rays much as do wood-rotting fungi. It can be found as abundantly in tangential as in radial sections. The hyphae are constricted as they pass

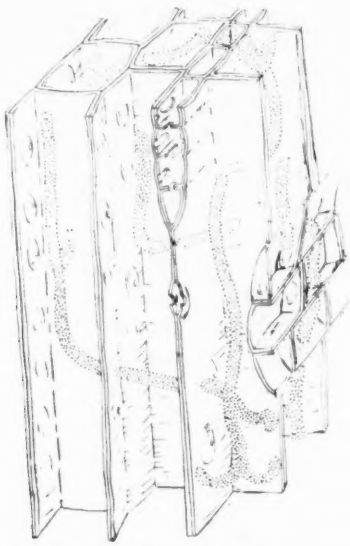

FIG. 1. Diagrammatic view of the bluestaining fungus in the heartwood of northern white cedar.

through the cell walls. Its growth in wood is shown diagrammatically in figure 1. Hyphae of the associated fungus or fungi are somewhat less abundant than those of the stain fungus, but can be seen readily and grow through the wood in a similar way.

Although this stain fungus has not been observed to produce spores during several years in culture on various agar media and on wood, it can be disseminated rather readily by hyphal fragments. About 5 cc. of sterile distilled water were added to a culture 4 months old on an agar slant in a test tube. This was shaken twice, then poured over malt agar in 6 Petri dishes, the excess water being poured off, so that only little of the original 5 cc. remained on the agar. Approximately 1000 colonies of the fungus arose on the six plates. Attempts to dislodge similar fragments by merely shaking old cultures without adding water, or by inverting them above agar, were unsuccessful.

The stain fungus, with the associated organisms mentioned above, was obtained in about 20 per cent of several hundred isolations from a brown cubical trunk rot that originates at branch stubs, and from a brown feathery butt and trunk rot that originates in the roots, of northern white cedar. The same bluestain is found very commonly in branch stubs of northern white

cedar and balsam fir and the same combination of organisms has been isolated from them repeatedly. What appears to be the same fungus has been observed in numerous samples of heartwood of western red cedar and Port Orford cedar.—C. M. CHRISTENSEN and F. H. KAUFERT, Division of Plant Pathology and Botany, Division of Forestry, University of Minnesota.

Cercosporella Eyespot of Kentucky Bluegrass.[1]—An undescribed disease, eyespot of Kentucky bluegrass (*Poa pratensis* L.), caused by a species of *Cercosporella,* occurs scatteringly in the Willamette Valley, Oregon. It was noted and specimens collected first at Forest Grove in March, 1939, and again a year later at East Corvallis. Strangely enough, although the symptoms are distinct, no specimens of it occur in any of the numerous earlier collections on this species of grass filed in the Mycological Herbarium of the Department of Botany at Oregon State College.

The writer has collected many specimens of *Septoria macropoda* Pass. var. **septulata** (Gz. Frag.) comb. nov. (*S. poae-annuae* var. *septulata* Gz. Frag.) on *Poa pratensis,* but in none of these does any of the cercosporella eyespot occur. It will be of interest, therefore, to note if this disease continues to spread under the cool, humid climate of mid-early spring in western Oregon.

On *Poa pratensis* the cercosporella eyespot is distinguished from the septoria leaf-spot and also from the leaf spot caused by *Helminthosporium vagans* Drechsl. as follows:

1. Cercosporella eyespot, lesions circular to sometimes finally elongate, brown with yellowish border, often straw colored in center and hence having an eye-spot type of lesion on at least some of the spots.
2. Septoria leaf spot, lesions variable in shape, rather uniformly ashy to stramineous in color, not an eyespot; pycnidia present.
3. Helminthosporium leaf spot, lesions circular to elongate with prominent, reddish-brown borders, darkening to nearly black in the center and, later, some becoming lighter in center.

The *Cercosporella* on *Poa pratensis* differs distinctly from any described species on Gramineae. Since the only possibility appeared to be the ill-described *Cercospora poae* Baudys et Picb., the writer sent material to Charles Chupp who very kindly examined it and replied that *C. poae,* in all probability, was the fungus known sometimes as *Scolecotrichum graminis* Fckl., and that the Oregon material probably was an undescribed species of *Cercosporella.* Chupp also suggested that it might be well to check on *Napicladium graminum* Peck. Material of this, furnished by H. D. House from the New York State Museum at Albany, also proved to be *Scolecotrichum graminis.* Therefore, *N. graminum* Peck can be added to the synonomy of *S. graminis* Fckl.

It appears, therefore, that the *Cercosporella* on *Poa pratensis* from Oregon is an undescribed species as follows:

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, and Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published with the approval of the Director of the Oregon Experiment Station as Tech. Paper No. 393. Contribution from the Department of Botany.

Cercosporella poagena sp. nov.

Maculis brunneis, margine aureo, ellipticis v. orbiculatis, deinde elongatis brunneis v. stramineis; conidiophoris hyalinis, obscuris; conidiis elongatis, sub-filiformibus basis obtusatis, apicibis acuminatis v. attenuatis, 4–7 septatis, hyalinis 45–90 × 3.6–5 μ (fig. 1).

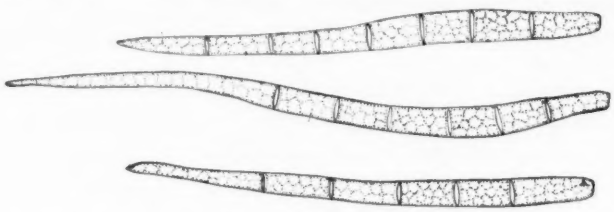

FIG. 1. Conidia of *Cercosporella poagena* sp. nov., from *Poa pratensis*, Forest Grove, Oregon (O.S.C. 453, type). × 1,000.

Hab. in foliis vivis *Poae pratensis*, Forest Grove, Oregon, March 15, 1939 (O.S.C. 453, type, filed at Oregon State College, Corvallis, Oregon, and a fragment of it in the Mycological Collections, Bureau of Plant Industry, Washington, D. C.). Also collected at East Corvallis (O.S.C. 789).

Lesions light-brown with a distinctive straw-color center and a surrounding border or halo of yellow; lesions at first very small, circular, later enlarging to nearly the width of the leaf or elongating longitudinally on the leaf for some distance; spots finally becoming dull brown and eventually fading to straw color. Conidiophores hyaline, obscure, arising from hyaline thinly formed aggregating hyphae; conidia hyaline, elongate, broadly filiform to obclavulate, bases blunt, tapering, apices either abruptly acuminate or attenuate at the tips, 4- to 7-septate (mostly 4-septate), 45–90 × 3.6–5 μ (45–75 × 3.6–4.6 μ in type and 57–90 × 4–5 μ in East Corvallis material).

On living leaves of *Poa pratensis*, Forest Grove (type) and East Corvallis, Oregon.—RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Northern Great Plains Field Station, Mandan, North Dakota.

Some Pathogenic Fungi Occurring in the Seed of Red and Subterranean Clover.[1]—In attempts to obtain red clover (*Trifolium pratense* L.) seedlings free of fungi, it was found that surface-sterilized seed often gave rise to fungi of known pathogenicity for clover. It seemed desirable, therefore, to determine the presence and prevalence of these fungi in the seed of red clover.

Thirty-four lots of red clover seed less than 1 year old, 15 lots 2 or more years old, and 6 lots of subterranean clover seed were tested. These seed lots were surface-sterilized by immersion in 95 per cent alcohol for 1 minute, a 1–1000 aqueous solution of bichloride of mercury for 7 minutes, and a saturated solution of calcium hypochlorite until transferred to potato-dextrose-agar plates. Notes were taken 10 to 21 days later. A total of 28,161 red clover seeds and 3,549 subterranean clover seeds were plated. Of the 34 lots of red clover seed less than 1 year old, 19 were infected with *Pleospora herbarum* (Pers. ex Fr.) Rabh. (*Stemphylium botryosum* Wallr.), 6 with *Stemphylium sarcinaeforme* (Cav.) Wilts., 1 with *Cercospora zebrina* Pass., and 2 with a sterile black fungus of large mycelium. The latter fungus,

[1] Contribution No. 33 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

first reported from Kentucky,[2] causes what is called the "black patch" disease of red and white clovers. This fungus has been reported also by Smith.[3]

The prevalence of these fungi under conditions of the tests was not great, the highest in any lot being 2 per cent with respect to *Pleospora herbarum*, and less than 1 per cent with the other fungi. It was necessary to plate out 1,635 seeds of one lot to demonstrate the presence of the "black patch" fungus in 2 seeds. Only *Pleospora herbarum* and *Stemphylium sarcinaeforme* were demonstrated in viable seed. Other fungi isolated were species of *Alternaria, Phoma, Fusarium, Penicillium, Oospora,* and *Chaetomium.* No fungi were found in the 15 seed lots 2 or more years old.

TABLE 1.—*Blemished and blighted seed of 5 lots of red clover, separated from macroscopically sound seed, surface-sterilized, and plated on agar. (Note the much higher incidence of fungi in the blemished seed)*

Condition of seed	No. seeds tested	No. seeds infected	Number of seeds with		"Black patch" fungus	*Pleospora herbarum*	Other fungi
			Alternaria sp.	*Stemphylium sarcinaeforme*			
Sound	2,225	32	26	0	0	0	6
Blemished and blighted	2,450	313	271	6	6	1	40

Of the fungi shown in Table 1 *Pleospora herbarum*[4] and *Stemphylium sarcinaeforme*[5] have been reported previously from red clover seed. It is possible that the introduction of the "black patch" fungus into new fields occurs through the seed, as no spores of this fungus are known to occur.

Isolations were made also to determine what fungi were present in the seed of subterranean clover (*Trifolium subterraneum* L.). From one lot of 980 seeds, *Sclerotium bataticola* Taub. was isolated 4 times, *Rhizoctonia solani* Kuehn once, and *Fusarium* 5 times. No fungi were isolated from 5 other lots of seed. The results of this preliminary test are included here, since the fungi isolated are known to be pathogenic on other crops.—ST. JOHN P. CHILTON, formerly with the U. S. Regional Pasture Research Laboratory, State College, Pa.; now with the Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University, Baton Rouge, La.

[2] Forty-sixth Annual Report of the Kentucky Agricultural Experiment Station (1933): (1) 1–69. 1934.

[3] Smith, O. F. A leaf spot disease of red and white clovers. Jour. Agr. Res. [U.S.] 54: 591–599. 1937.

[4] Smith, O. F. *Stemphylium* leaf spot of red clover and alfalfa. Jour. Agr. Res. [U.S.] 61: 831–846. 1940.

[5] Krakover, L. J. The leaf-spot disease of red clover caused by *Macrosporium sarcinaeforme* Cav. Rept. Mich. Acad. Sci. 17: 273–328. 1917.